

Center for Bioenvironmental Research

Olitesof Research Annual Productivity Report 2001-2002

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Center for Bioenvironmental Research At Tulane and Xavier Universities Bioenvironmental Hazards Research Program Office of Naval Research/US Department of Defense Annual Productivity Report N00014-99-1-0763

ABSTRACT

Beginning in April 1999, the Center for Bioenvironmental Research (CBR) at Tulane and Xavier Universities has received funding from the Office of Naval Research to continue its Bioenvironmental Hazards Research Program (BHRP). This funding has supported a suite of complementary research projects that address the impacts of bioenvironmental hazards on environmental signaling from molecular to ecosystem levels and makes connections between these impacts. The research ranges from basic research on proteomics to applied technology development of biosensors and autonomous underwater vehicles for monitoring. The BHRP program includes mechanisms for the effective communication of this information for resolution of Department of Defense problems and for the educational training of future scientists.

One module, Environmental Signals and Sensors, utilizes basic research on how chemical signals on molecular, cellular, and organismal levels can be utilized for assessments of human, wildlife, and plant health; and development of biosensors for assessments of toxicity and risk. Areas of focus, including human and ecological health, integrate research themes in this module by extending environmental signaling to human health endpoints at individual and population levels, or extending to ecological and ecosystem function levels. Given the CBR's and Navy's mutual interest in biosensors and brown water/ocean systems, a second module, Ecosystem Monitoring and Assessment, has placed special research emphasis on the small scale turbulence of the model ecosystem of the Mississippi River/Gulf of Mexico and the development of biosensors and autonomous platforms for ONR and DOD applications. As a suite of integrated modules, specific projects thus have complemented each other as part of a holistic BHRP to aid in effective and comprehensive environmental assessments for the DOD.

Fourteen research projects have been conducted in the two primary research modules and have resulted in significant progress related to the overall grant objectives. Assisting in the implementation of the overall project to promote the dual resolution of DOD problems and education of students and the general public was the continuation of four support cores:

1) environmental informatics; 2) computer operations; 3) research support; and 4) communication and education. In addition to the new knowledge developed by the research effort, the program has produced 3 useable technologies for further development, 71 publications and abstracts, 50 presentations, and supported the intellectual development of 23 graduate students, 8 undergraduates, and 6 CBR SPRITE students.

This program reflects the CBR's existing research strengths and employs a set of complementary, integrated research modules to assess the impacts of "environmental signals" (e.g., contaminants and pollutants) on humans and ecosystems. The integration of all the research modules has resulted in a comprehensive program of environmental research that provides the ONR with a technology package that spans research initiation to communication of environmental findings to appropriate target audiences. Transcending traditional structures, the CBR has become a model of academic/government/industry interaction.

List of Acronyms and Abbreviations

AUV autonomous underwater vehicles

BHRP Bioenvironmental Hazards Research Program

CBR Center for Bioenvironmental Research

COAMPS Coupled Ocean Atmosphere Mesoscale Predication Systems

COTS Commercial off-the-shelf

DES diethylstilbestrol

DNA deoxyribonucleic acid

EARS Environmental Ambient Recording Systems

EDCs endocrine disrupting chemicals
EDTA ethlenediaminetetraacetic acid

EEB Ecology and Evolutionary Biology (formerly EEOB)

EEE Eastern Equine Encephalitis

EPA Environmental Protection Agency

ER estrogen receptor

GC/MS gas chromatography/mass spectrometry

GIS Geographical Information System

GPS Geographic Positioning System

LBM Lattice-Boltzman Method

LEAG Long-Term Estuary Assessment

MCB Molecular Cell Biology
MD Molecular Dynamics

MoA Memorandum of Agreement

NAVOCEANO Naval Oceanographic Office

NRL Naval Research Laboratory

PAH polyaromatic hydrocarbons

POC particulate organic carbons

PPCP pharmaceutical and personal care products

QSAR Quantitative Structure Activity Relationships

REMUS
Remote Environmental Monitoring Underwater System

SPRITE
Summer Pipeline Research Initiative: the Tulane Experience

TGF-β1 transforming growth factor beta

TNF-α tumor necrosis factor alpha

TUHSC Tulane University Health Sciences Center

UV ultra violet

XU Xavier University

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Center for Bioenvironmental Research At Tulane and Xavier Universities

Bioenvironmental Hazards Research Program Office of Naval Research/US Department of Defense

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I. OBJECTIVES AND SIGNIFICANCE

The Office of Naval Research (ONR) is interested in long-range science and technology research projects that offer potential for advancement and improvement of Navy and Marine Corps operations and encourages participation by Historically Black Colleges or Universities (HBCUs). This final technical report describes an integrated program of basic and applied bioenvironmental research for technology development, communication and education that supports the ONR Bioenvironmental Hazards Research Program (BHRP). The work described in this report builds upon the DOD's nine-year, integrated research program (BHRP) conducted at the Center for Bioenvironmental Research (CBR) at Tulane and Xavier Universities. The CBR focuses on the holistic concept of environmental signaling from molecular to ecosystem levels, with a particular emphasis on development of biosensors, biomarkers, and evaluation techniques related to environmental exposures of human and ecological systems that addresses bioenvironmental problems relevant to the Navy and the Department of Defense (DOD). CBR research taps the basic and applied strengths of two universities that have been directed and refined over a nine-year period to reflect and, indeed, anticipate DOD environmental research interests.

A. Environmental Priorities of the Office of Naval Research

Many of the DOD environmental programs seek to understand the fate and biological effects of pollutants and contaminants resulting from military operations and training. To achieve this goal, DOD has focused on basic research to understand the biological actions of these agents, biomarkers of exposure, mechanisms of toxicity, and the use of experimental and computational modeling to assess potential health risks. The ONR focuses on "brown water" problems of particular Navy relevance (e.g., major world rivers, estuaries, and continental shelves). Of particular interest to the ONR Biomolecular Science and Technology Program are 1) biomolecular projects related to biosensor development; and 2) biosystems projects related to marine mammal biology and environmental microbiology/engineering.

Central to meeting these challenges is the continued development of new knowledge, technology, and human resources through the nation's universities, where approximately half of the defense science and technology research is currently performed. The ONR is committed to strengthening the scientific capability of colleges and universities with significant enrollments from minorities underrepresented in science and engineering, and providing science-related infrastructure as well as funding for defense research and engineering programs. Support of the nation's university-based science and education enterprise is an essential component in addressing environmental concerns facing DOD.

The CBR submitted a proposal in response to the Broad Agency Announcement (BAA 00-018). In January 2001, the Office of Naval Research awarded the Center for Bioenvironmental Research (CBR) at Tulane and Xavier Universities a one-year renewal grant of \$1.9 million in funds for the Bioenvironmental Hazards Research Program (BHRP), beginning in May 1, 2001, with a no-funds extension in April 2002 extending the period of performance through September 30, 2002. This funding has supported a suite of complementary research projects that address the impacts of bioenvironmental hazards on environmental signaling from molecular to ecosystem levels and makes connections between these impacts. The research ranges from basic research on proteomics to applied technology development of biosensors and autonomous underwater vehicles for monitoring. This annual productivity report also includes mechanisms for the effective communication of this information for resolution of DOD problems and for the educational training of future scientists.

B. Prior Progress of the CBR Biohazards Research Program

The CBR BHRP to date has developed an integrated approach to increasing the knowledge base of actual and potential impacts on human health and ecological systems of defense-related operations, as well as the processes to restore contaminated environments. To facilitate the long-term security of the Navy, other military services, and the nation, the CBR BHRP has:

- Produced a vast suite of technologies and methods for biohazard monitoring and characterization of ecosystem, wildlife, and human health for application biohazard risk assessments for all of the DOD's branches;
- Developed one of the world's most robust programs for biosensor and biomarker technology development for real-time cost-effective monitoring of heavy metal and organic contaminants and combat-related biohazards in the air, water, and the soil including one deployed on an autonomous underwater vehicle (AUV);
- Facilitated a strategic partnership between academia, military, agency, and the commercial sector in the Gulf South region for a holistic long-term monitoring program for the Mississippi River, the Gulf of Mexico, and airshed to serve as a national testing laboratory for military biohazard monitoring, characterization, and communication;
- Increased the nationwide representation of African Americans with advanced degrees in bioenvironmental fields important to DOD; and
- Funded more than 100 projects over the past decade, including those through the ONR, resulting in hundreds of publications, collaborations, and investments with the commercial sector.

These projects are being conducted through a wide variety of methods including *in vitro*, *in vivo*, epidemiological, modeling, field, and other laboratory studies at Tulane and Xavier Universities and associated sub-contractual institutions. The significance of this research includes a greater understanding of human and ecosystem responses to environmental contamination and their ability to repair or reverse these effects; increased safety for defense workers and the general public from exposure to toxic exposures; and technologies for advanced monitoring of the environment for the improvement of human and ecosystem health.

An additional benefit of the CBR BHRP is the education and research training provided to minorities. Past research activities have trained both minority faculty and students at Xavier University in scientific analytical techniques and have increased minority representation in these fields. These are techniques applicable to a variety of environmental concerns, but of particular relevance to ONR problems.

The most important result of the CBR BHRP is the development and practical application of basic knowledge and new technologies. BHRP technologies have been demonstrated through numerous environmental applications in both defense and private sectors. Through its research, the CBR has attracted corporate investments including: \$1 million from Shell, \$75,000 from TRW, and other investments from Uniroyal and Exxon. Many of these BHRP projects have demonstrated either existing or potential commercial and civilian interest or partnerships. The useable technologies that have demonstrated the greatest commercial and civilian interest to date are summarized in **Appendix C**.

C. Current Research Efforts of CBR Biohazards Research Program

This program reflects the existing research strengths of the CBR and employs a set of complementary, integrated research modules to assess the impacts of "environmental signals" (e.g., contaminants and pollutants) on humans and ecosystems. The integration of all the research modules has resulted in a comprehensive program of environmental research that provides the ONR with a technology package that spans research initiation to communication of environmental findings to appropriate target audiences. There are few, if any, academic organizations with this capability.

One module, Environmental Signals and Sensors, utilizes basic research on how chemical signals on molecular, cellular, and organismal levels can be utilized for assessments of human, wildlife, and plant health; and development of biosensors for assessments of toxicity and risk. Areas of focus, including human and ecological health, integrate research themes in this module by extending environmental signaling to human health endpoints at individual and population levels, or extending to ecological and ecosystem function levels. Given the CBR's and Navy's mutual interest in biosensors and brown water/ocean systems, a second module, Ecosystem Monitoring and Assessment, has placed special research emphasis on the model ecosystem of the Mississippi River/Gulf of Mexico and also the development of biosensors and autonomous underwater platforms for ONR and DOD applications. Cores that provide research support include computer operations, environmental informatics, and communication and education to promote the dual resolution of DOD problems and also the education of students and the general public. As a suite of integrated modules, specific projects thus have complemented each other as part of a holistic BHRP to aid in effective and comprehensive environmental assessments for the DOD.

II. CBR Capacity

A. Introduction to the CBR

The mission of the Center for Bioenvironmental Research (CBR) is to conduct and coordinate research and teaching to enhance global understanding of environmental

issues and provide solutions through innovative communication and technology. Founded in 1989, the CBR is an innovative and effective partnership between a Historically Black College or University (HBCU) and a major research university that encourages scientists from multiple disciplines to work together to investigate and resolve environmental problems.

Under the leadership of Dr. John McLachlan, Weatherhead Distinguished Professor in Environmental Studies at Tulane University and an internationally recognized environmental scientist and administrator, the CBR has earned a reputation for its scientific research into the environmental problems of Louisiana. In extending its spheres of influence to national and global problems of the environment, the CBR has brought unique focus that reflects a community-based perspective in conjunction with scientific rigor.

CBR programs are organized around five themes:

Partnerships: The CBR integrates faculty and students from Tulane's Schools of Engineering, Liberal Arts and Sciences, Medicine and Public Health and Tropical Medicine and Xavier's Colleges of Arts and Sciences and Pharmacy in innovative ways to optimize interdisciplinary teaching, learning and research.

Human and Ecosystem Health: Integrating diverse disciplines, the CBR has developed a holistic research program focusing on the effects of environmental hormones on humans and ecosystems through the processes of environmental signaling by natural and synthetic hormones and contaminants that mimic those substances.

Water: The CBR has co-evolved its programs on Environmental Signaling and Aquatic Ecosystem Research to create effective connections. Research efforts employ laboratory and field-scale approaches to look at physiochemical, biological, and ecological impacts in the Mississippi River, Gulf of Mexico, and other aquatic and atmospheric ecosystems.

Communication & Technology: The CBR provides research-based knowledge on the origins, interactions and fate of natural and synthetic chemicals in living systems using informatics capacity particularly its data management and GIS teaching lab. Through its web-based information programs and networked digital technologies, the CBR makes complex issues understandable and provides a forum for scientific discourse.

Environmental Education: The pipeline programs provide interdisciplinary training and research opportunities for undergraduate, graduate and doctoral students and faculty. Internet-based educational programs and outreach initiatives strengthen science education on campus and in the local community and region.

B. The CBR Tulane/Xavier Partnership – a Unique Model

Tulane and Xavier Universities have developed a close working relationship in the past thirteen years, aided by a common vision of academic excellence and the development of high quality educational opportunities for minorities and women. The Tulane/Xavier partnership is a well-established, effective joint venture between majority and minority

universities. It is an extremely complementary relationship with respect to environmental restoration and waste management, with the Xavier University focus on education and graduate work, and the Tulane University emphasis and experience in education, research, and technology development and transfer. Optimizing the research capabilities of Tulane and the educational resources at Xavier, the reputation of both institutions enhances the ability of the CBR to solicit resources, recruit staff and researchers, sponsor conferences, and execute successful marketing of its education and research programs.

The relationship between Tulane and Xavier Universities is the foundation of the CBR and serves as a working model for all its collaborations. Since the CBR integrates academic structures, it has the freedom to advance teaching and research by creating flexible working groups to address specific needs and problems. Administrators and researchers team up with government, private, academic, and community individuals and agencies to make use of the best intellectual and technological resources. The partnerships catalyzed by the CBR exist at local, regional, and international levels resulting in community-based solutions to environmental health problems.

1. Tulane Capabilities

Tulane University has established itself as a powerful engine of economic development for New Orleans and Louisiana. Beyond that, in its 160-year commitment to education, Tulane has developed itself as a good citizen that provides the spark of creativity and knowledge that attracts and nurtures intellectual talent while directing its resources to the needs of the community. Tulane is the largest private employer in Orleans Parish and ranks 12th in the State of Louisiana.

Since its inception Tulane has grown into one of the nation's premier institutions of higher learning, known widely for both its undergraduate teaching and cutting-edge research. Those achievements are reflected in rankings by national periodicals such as *US News and World Report* that in 2003 ranked Tulane 44th overall among all national universities. Tulane University ranks 15th among private universities in income received from technology transfer.

The University enrolls a diverse student body of 13,000 students from all 50 states and 110 foreign countries in its undergraduate, graduate and professional schools. The University ranks in the top 10 in Environmental Law Studies, the top 15 in Public Health and International Law, and the top 25 in Biomedical Engineering. Tulane is recognized as both a member of the Association of American Universities and a Carnegie-1 research university, one of only 4 in the South.

2. Xavier Capabilities

Xavier University of Louisiana is the nation's only institution of higher education that is historically African American and Catholic. The ultimate purpose of the University is the creation of a more just and humane society. To this end, Xavier prepares its students to assume roles of leadership and service in society. This preparation takes place in a pluralistic teaching and learning environment that incorporates all relevant educational means, including research and community service.

Xavier University has grown in enrollment in the past 10 years with a current enrollment of 4,000 students. More than half of the Xavier students are from Louisiana, while the rest come from 41 other states, the District of Columbia, the Virgin Islands, and 14 countries. The student body is predominantly African American (85%), but the university is open to all. More than half of its students currently major in the natural or health sciences.

The preeminence of Xavier in training undergraduates in science is reflected in its first-place ranking in the placement of African-American graduates in medical schools for the past 10 years. In addition, Xavier is first nationally in the number of African American students earning undergraduate degrees in both biological/life sciences and the physical sciences. Nearly 50% of all recent graduates enroll in professional and graduate schools. The National Science Foundation has designated Xavier University as one of six to participate in its "Model Institutions for Excellence in Science, Engineering, and Mathematics" program. The Southern Association of Colleges and Schools accredits Xavier University.

C. CBR Support Cores

The state of the art CBR facility at Tulane Health Sciences Center contains sophisticated research equipment including exposure chambers for respiratory disease studies and a Geographical Information Systems (GIS) lab. The facility is electronically networked and physically connected to Tulane Medical School and University Hospital, thus providing easy access between laboratories and clinics. The CBR facility contains state-of-the-art research equipment including a microarray core facility, a fluorescent activated cell sorter, and a high performance gas-liquid chromatograph/dual mass spectrometer. In terms of molecular biologic capabilities, the labs also feature analytical devices, and cellular and molecular biology equipment, tissue-culture facilities, and various containment and decontamination hoods and devices, so that radiation research, protein analysis, PCR, RNA and DNA analyses and immunohistochemical and in situ hybridization procedures are routinely conducted in the course of experimentation.

The CBR also has research space on the Tulane University uptown campus. The CBR Uptown contains an additional GIS lab and many equipment cores and lab space for studying neuroscience, molecular biology, and analytical chemistry. The CBR faculty located at Xavier University utilizes modern research and office space in the new seven-module environmental toxicology research center.

1. Research Support

The CBR has a first-rate management team in place with administrative capabilities to develop, implement, facilitate, track, and monitor grants and contracts as well as provide programmatic direction and administrative leadership for the facility. This team includes a director, a deputy director, associate directors, program managers and coordinators, accountants and administrative secretaries. Tulane and Xavier are innovators among universities in facilitating the mechanisms that allow scientists from multiple disciplines to work together in resolving environmental problems. In addition, the organizational structure of the CBR has allowed it to qualify for large integrated, interdisciplinary grants

that are beyond the scope of many other universities or research organizations, including individual Tulane and Xavier departments. The work of the CBR is strengthened by this partnership that can provide the faculty, students, and resources that are necessary to conduct the bioenvironmental hazards research projects discussed in this report.

2. Computer Operations

The CBR has established a Computer Operations Core to modernize and facilitate efficient data communication, sample tracking, QA/QC, and data dissemination. Presentation capabilities are enhanced by a state-of-the-art computer operations facility that provides graphics and electronic media-based services and lab-based microscopy with a digital camera and PC with Image Pro archiving and image analysis software.

The Center provides fast, accurate access to the results generated by high tech analytical instruments located in various Tulane and Xavier scientific laboratories. The SGI equipment is primarily utilized for molecular modeling and other supercomputing tasks that require symmetric multiprocessing and geometry engines for advanced 3-D rendering. Apple and PC notebooks enable the CBR staff to communicate with the department by establishing a link for Internet access, electronic mail, and data transfers while attending events away from the home base. In addition to the equipment currently maintained at satellite locations, the CBR has shared access to Tulane University distributed computing environment of high performance RISC computers. These machines enable the Tulane community to stay connected with other institutions that are part of Internet 2 at data transfer speeds in excess of 100 Mbit/sec as well as other Internet entities at 10Mbit/sec.

During the ONR project period, the Computer Operations Core established and maintained the IT infrastructure necessary to accommodate all project requirements for analysis and information exchange. Collaborative efforts also resulted in the establishment of multiplatform wireless connectivity. As part of the effort to maintain these services the Computer Operations Core acquired various hardware and software, implemented networking protocols, managed web-based information exchange and maintained data warehousing and safekeeping.

3. Environmental Informatics

During the ONR project period, the Environmental Informatics Core provided spatial (GIS) analysis, remote sensing, mapping, database development and online systems development in collaborative projects with BHRP researchers. Specific ONR research focused on three areas:

- A detailed and ongoing analysis of the historical bathymetry of the lower Mississippi River during the 20th century resulted in the discovery of significant patterns of aggradation and erosion in the lower Mississippi River.
- Research on the habitat characteristics of the *Aedes aegypti* and *Culiseta melanura* mosquitoes, utilizing GIS technologies, resulted in the identification of statistically significant correlations between species occurrence and habitat types.
- Mapping and cartographic work in relation to human health research and Mississippi River studies was produced.

 A large database of GIS and remote sensing datasets of southeastern Louisiana has been amassed.

4. Communication and Education

Funds were allocated from the Research Support core to cover student support for research teams. In this way the CBR could assist research faculty to build and train sufficient qualified personnel to complete research and also create capacity in undergraduate students. Funds were also provided for three projects in the Communication and Education core for training of students through educational initiatives. They were: 1) two education pipeline pilot programs; and 2) the annual international symposium of presentations, posters and workshops on environmental signaling (the e.hormone conference).

Education Pipeline Pilot Programs: With ONR support, the CBR continued the successful SPRITE program as part of its undergraduate education pipeline initiative to increase the number of African American students enrolling in graduate science programs. The Summer Pipeline Research Initiative: the Tulane Experience (SPRITE) provided Xavier undergraduate students a graduate-level laboratory research experience and mentored introduction to graduate life. In three years, this program has become a major pipeline of minority students to Tulane graduate and health professional schools. Of the 6 students who completed the 2001 summer program, 2 were accepted to medical schools (one to Tulane, and the other to the University of Chicago, Medical Schools), one was accepted into Cornell University Graduate School, another graduated and has taken a year off from academics, and the remaining 2 are finishing their final 2 undergraduate years. One of these students has been accepted into Tulane Medical School through an early admissions program.

A second pilot education pipeline component, a joint degree program between Xavier Chemistry/Biology Departments and Tulane Engineering, has as its intent the production of minority graduates with undergraduate degrees plus MS degrees in engineering. Initiated in Fall 2000, three Xavier students began their fourth (undergraduate) year by coenrolling at Xavier and Tulane Universities. A summer of research followed by a full academic (5th) year at Tulane fulfills course requirements for the master's degree as well as the undergraduate degree. The original 3 students will graduate in 2003, 3 more students have joined the program, and 2 new applications are under review. Programmatic changes include the addition of biomedical engineering as an elective field. Most importantly, the Tulane/Xavier education pilot funded by ONR has been institutionalized and formally recognized through an articulation agreement signed by the Presidents of both universities.

International Environmental Hormone Symposia: One of the central themes of the CBR's Integrated Bioenvironmental Hazards Research Program is understanding how bioenvironmental contaminants can impact the health of humans and wildlife and their progeny through disruption of the endocrine system. Understanding the many issues surrounding environmental endocrine disruption, or environmental signaling (eg. contaminants and pollutants) and its effects on human and ecosystem health requires a synthesis of disciplines ranging from molecular biology to systemic population biology. For the past three years, the annual Environmental Hormone Symposium (e.hormone) in

October initiated and hosted by the CBR has been a national and international focal point for all those who are interested in the field of environmental signaling.

The goal of the *e.hormone* symposium series is to bring together innovative thinkers, cutting edge researchers, and key decision makers to critically evaluate current research on environmental signalling and contribute to the future of this emerging field. The symposium format includes scientific presentations grouped around conceptual themes. Preeminent experts in the field introduce sessions and provide historical perspective on their topic and highlight recent findings. Presentation topics range from human to ecosystem health and from basic research to population studies and all explorations were at the cutting edge of research and policy. The primary strength of this annual forum of information exchange and collegial interaction is its multidisciplinary and multinational nature. Each of the past three symposia has been reported on the web, and its scholarship recognized in publications such as *Science News* and *the Annals of the New York Academy of Sciences*. e.hormone 2001 was host to 39 speakers (10 international) and 210 participants (37 international).

In summary, the CBR is dedicated to training students for careers in science. The CBR sponsors numerous programs to increase and enhance undergraduate, graduate, and faculty research and training opportunities at Tulane and Xavier Universities. A primary CBR goal is to encourage and enhance minority participation and representation in the sciences. The Communication and Education core reflects a collection of enriching environmental education programs that promote awareness of pertinent issues, offer interactive encounters between young and veteran scientists, and provide career-building research experiences in bioenvironmental, biomedical, and environmental sciences.

III. OVERVIEW OF RESEARCH

A. Introduction

Research associated with environmental problems of importance to the DOD has required an integrated approach from fundamental science to communication of research results. This report provides a set of integrated research modules that will continue the CBR/ONR BHRP partnership and serve as a model for other DOD research along environmental lines. The CBR has earned a reputation for its scientific research into relevant environmental problems, while becoming a model of academic/government/industry interaction. Since the CBR transcends traditional academic structures, it provides a powerful tool for modernization of teaching and research.

These research projects integrate common research themes of environmental signals and ecosystem monitoring as well as common technologies of biosensors or biomarkers. Computer Operations and Environmental Informatics cores ensure integration of this research in conjunction with Communication and Education core, which elucidates communication of this research and the flow of information to future scientists and engineers. One of the benefits of this modular approach is the applicability of these approaches to environmental problems using research themes or technologies that are common across platforms. An additional

benefit of these reports is the provision of a mechanism for the bi-directional flow of initiatives and insights from the Navy to and from academia, while providing for the education of future scientists.

In the area of Environmental Signals and Sensors, four projects were funded. Of these four, one was a joint Xavier/Tulane effort, led by the Tulane investigator. In the area of Ecosystem Monitoring and Assessment, ten projects were funded. Of these ten projects, Xavier investigators led two. In the Communication and Education area, three projects were funded. One was a joint Tulane/Xavier effort. A total of seventeen (17) projects were funded under the CBR BHRP award of May 2001.

B. Environmental Signals and Sensors

A principal goal of our ongoing research is to examine the actions of DOD-relevant contaminants/pollutants (e.g., organo-chlorine compounds, PAH's, and heavy metals) on important cell targets. Long-term goals of the research in this module are to identify suitable biological markers that will serve as early indicators of toxicant exposure in humans and wildlife and potentially of the overall health of the ecosystem, thereby linking with the other research module in this project. The research addressing this theme will elucidate a scientific basis to develop rational biologically-based risk assessment models.

The projects related to this theme apply molecular biology to elucidate mechanisms of toxicity of these signals and to develop new methods for sensing of toxicants in the environment. Understanding the mechanisms of these toxic reactions permits the development of real understanding of the hazard posed by various contaminants. The data derived from these projects will yield scientific bases to assess risk to humans and wildlife.

In the area of Environmental Signals and Sensors, four (4) projects were funded.

- Dr. Diane Blake, Associate Professor of Ophthalmology, Tulane University, with Co-Investigators Dr. George Flowers, Associate Professor of Geology and Chair, Tulane University, and Dr. Robert Blake, Professor and Interim Chair of Basic Pharmaceutical Sciences, Xavier University, used a set of high-affinity, highly selective binding reagents to develop an immunosensor for EDTA that can operate in an autonomous underwater vehicle ("Antibody-Based Biosensors for Autonomous Underwater Vehicles").
- Dr. Matthew Burow, Assistant Professor of Medicine, with Co-Investigator Dr. John McLachlan, Professor of Pharmacology, Tulane University, is developing technologies and methods to identify a basic mechanism by which selected environmental agents subvert the estrogen and cell survival signaling pathways, thereby leading to potential developmental defects and/or disease states ("Effects of Estrogens and Endocrine Disrupters on Suppression of Apoptosis in Normal and Neoplastic Breast Epithelial Cells").
- Dr. Scott Michael, Assistant Professor of Tropical Medicine, Tulane University, is determining whether frozen sperm from an extinct species are able to direct development of enucleated eggs from a closely related extant species ("Ecological Remediation: Resurrection of an Extinct Species of Frog from Puerto Rico").

Dr. Valerie Wilson, Clinical Associate Professor of Environmental Health Sciences, Tulane University, is studying genetic and biologic markers related to regulation of uterine function in women ("Effects of the Environment on Leiomyoma Cells and Women's Health").

C. Ecosystem Monitoring and Assessment

The Navy requires a fundamental understanding of fate, transport and transformation effects of contaminants in estuarine and near-shore environments. Since DOD operations can frequently result in the release of a variety of perturbations in a region, a holistic assessment of potential biohazard impacts must include ecosystem-level analyses. Through basic and applied research, the CBR is developing sensor devices that will monitor potential and actual exposure of troops in the field to harmful chemical or biological agents. CBR research emphasis is on providing innovative cost-effective solutions to prevent, minimize, or remedy human health or ecological hazards. CBR expertise on environmental characterization, monitoring, and assessment achieved provides an essential segue into practical research on appropriate environmental impact assessments and management based on that assessment. Research results will advance the current state of knowledge in remediation policies (including determinations of self-remediation) and can result in substantial cost savings for the DOD and other public and private entities in future environmental impact assessment efforts.

In the area of Ecosystem Monitoring and Assessment ten (10) projects were funded.

- Dr. Brent McKee, Professor of Geology, Tulane University, with Co-Investigators, Dr. Tom Bianchi, Associate Professor of Ecology and Evolutionary Biology, Tulane University, Drs. Mike Dagg and Rodney Powell, Louisiana Universities Marine Consortium, and Dr. Richard Miller, Chief Scientist, NASA Stennis Space Center, used remote sensing, biological and geochemical techniques to determine the major pathways of carbon in the Mississippi River ("River-Ocean Interactions (Phase I): The Processing and Fates of Nutrients and Organic Carbon from the Mississippi River").
- Dr. Douglas Meffert, Clinical Associate Professor of Environmental Health Sciences, Tulane University, with Co-Investigator George Rey, President of COTS Technology, pursued integration and technology co-development links to deploy biosensors in remote environments, specifically through autonomous/unmanned underwater vehicles ("Autonomous Monitoring and Visualization Technology Development for Aquatic Environments").
- Dr. Efstathios Michaelides, Professor of Mechanical Engineering, Tulane University, with Co-Investigators Dr. Laura Steinberg, Assistant Professor of Civil and Environmental Engineering, Tulane University, and Dr. Elia Eschenazi, Associate Professor of Physics and Engineering, Xavier University, studied the sedimentation and resuspension characteristics of single particles and flocs in aqueous solutions ("Sedimentation and Resuspension Studies for the Mississippi River and Louisiana Coastal Environments").
- Dr. David Sailor, Associate Professor of Mechanical Engineering, Tulane University, initiated research development efforts related to coastal urban

- applications of the COAMPS mesoscale atmospheric modeling system currently in use by the US Navy ("COAMPS for Coastal Urban Transport Studies Developing Infrastructure and Testing Models").
- Dr. Elia Eschenazi, Department of Chemistry, Xavier University, with Co-Investigator Dr. Kyriakos Papadopoulos, Tulane University, studied the stability of colloidal particles in diverse conditions so as to develop a generalized model for predicting the fate and transport of subsurface contaminants ("Roles of Natural Organic Matter (NOM) on the Transport of Colloidal Particles").
- Dr. Mead Allison, Department of Earth and Environmental Sciences, Tulane University, investigated the seasonal storage of sediment in the lower Mississippi River in Louisiana in order to determine the volumes, areas affected, and mechanisms of formation ("Acoustic Mapping of Sediment Storage in the Lower Mississippi and Atchafalaya Rivers").
- Dr. Kathleen McCloud, Associate Professor and Chair, Department of Physics and Engineering, Xavier University, with co-investigator Dr. Elia Eschenazi, Professor, Department of Physics and Engineering, Xavier University, studied the interaction between the hydrodynamics of sedimenting particles and the resulting roughness and growth of their surface ("The Effect of Particle Dynamics on Rough Surfaces Formed by Sedimenting Particles").
- Dr. Franco Marcantonio, Associate Professor, Department of Earth and Environmental Sciences, Tulane University, further defined the mechanisms of removal and/or labilization of strontium and other trace elements in the estuarine mixing zone of the Mississippi River ("Sr and Pb Isotope Studies of Mississippi Delta Estuarine Waters and Sediments").
- Dr Doug Meffert, Clinical Associate Professor, Department of Environmental Health Sciences, Tulane University, has overseen the use of the RV Eugenie by ONR researchers primarily for measuring river concentrations of natural and manmade chemicals in the Mississippi River and its related ecosystems ("RV Eugenie Field Project").
- Dr Doug Meffert, Clinical Associate Professor, Department of Environmental Health Sciences, Tulane University, with co-investigators Dr. Glen Boyd, Assistant Professor, Department of Civil and Environmental Engineering, Tulane University, and, Dr. Deborah Grimm, Director, Coordinated Instrumentation Facility, Tulane University, investigated the occurrence of pharmaceuticals and personal care products and endocrine disrupting chemicals in waters of southeastern Louisiana and potential effects on the drinking water community ("Occurrence and Fate of Pharmaceutically Active Compounds and Other Emerging Contaminants").

IV. SUMMARY ACCOMPLISHMENTS

A. Overview

Accomplishments on this grant can be documented in three major areas:

1. **Research Publications**, **Abstracts and Presentations** – that document progress made in the research through publication in a number of peer-reviewed venues available to the general scientific community;

- 2. **Development of Useable Technologies** which have direct benefit on furthering the mission-related scientific interests of ONR; and
- 3. *Intellectual Development* that extends the ability of the grantee and ONR by developing the next generation of scientists.

For final investigative research reports, see Appendix A.

Research Publications, Abstracts and Presentations:

Research from this project period resulted in the following publications in scientific journals and conference reports:

- Seventy (70) publications in research journals and in conference reports such as Biosensors and Bioelectronics, Endocrinology, Environmental Health Perspectives, Environmental Science and Technology, Geochimica et Cosmochimica Acta, International Journal of Oncology, Journal of Clinical Endocrinology and Metabolism, Journal of Comparative Endocrinology, Journal of Fluids Engineering, Journal of Geophysical Research, Journal of Plankton Research, Marine Chemistry, Marine Geology, Molecular Carcinogenesis, Nutrition and Cancer, Organic Biochemistry among others.
- One (1) published abstract in meeting and symposium reports.
- Fifty (50) presentations at major scientific conferences nationally and internationally including the annual meetings of the American Association for Cancer Research, American Society of Limnology & Oceanography, American Water Works Association, Geological Society of America, Gulf Coast Association of Geological Societies, and the National Ground Water Association.

A complete listing of the publications, abstracts and presentation made by investigators on this project can be found in Appendix B.

Development of Useable Technologies:

Research results from this project are generating three useable technologies.

One technology that is part of the Environmental Signals and Sensors area is:

• Immunosensor for AUV Deployment. This antibody-based biosensor will automatically collect and analyze 5 separate samples after installation in an autonomous underwater vehicle or immobilized buoy. A self-contained, automated immunosensor will have the capability to detect very low concentrations of environmental contaminants and/or chemical and biological weapons in surface waters. An assay that detects nanomolar levels of EDTA, the first analyte to be developed for this instrument, has been established. Transfer of the assay to the immunosensor will begin when Sapidyne has corrected the defects in the optical components of the instrument. Sapidyne Instruments (Boise, ID) is constructing the immunosensor and the Tulane laboratory, in conjunction with the Naval Research Laboratory (Dr. Fran Ligler, Washington, DC), is working closely with them to coordinate the development of biological reagents with the development of the instrument. A provisional patent application entitled "Recombinant antibodies that bind to metal-chelate complexes" was filed in March 2001.

The two technologies that are part of the Ecosystem Monitoring and Assessment area are:

- Integrated Autonomous Immunosensor & Autonomous Underwater Vehicle (AUV) System. This system will enhance real-time biosensor deployment for environmental compliance and ultimately biologic warfare detection. The projected timetable for the completion of AUV/ biosensor integration is August 2004, and the biosensor will be deployed on AUV or stationary buoys and AUVs subsequent to August 2004, pending acquisition of REMUS AUV. Partners are Tulane and Xavier Universities; COTS Technology, LLC; Sapidyne Instruments (ID); Woods Hole Oceanographic Institute (MA); and the US Naval Oceanographic Office (MS). Patents will be applied for by the partners.
- An urbanized mesoscale model. This product will involve several model enhancements to the existing version of the COAMPS mesoscale atmospheric modeling system currently in use by the Navy. Specifically, the unique modeling improvements developed for another leading mesoscale model (MM5 from the National Center for Atmospheric Research) will allow enhanced predictive capability for atmospheric flow and heat transfer in and around metropolitan regions. The urbanized model will be better able to capture the meteorological aspects of the urban heat island effect, and fate and transport of pollutants in urban domains. The EPA has an interest in improving the capabilities of models over urban areas as a means of improving the capabilities of photochemical air quality models.

Further details on the potential technology products can be found in Appendix C.

Intellectual Development:

The research effort provided for the intellectual development of the faculty who participated in the project. In the process of conducting their research, investigators collaborated with new and existing partners in research, and at times formed unique consortia and research teams. Several of the investigators worked across departmental boundaries and in a few instances, faculty members from each component university formed a Tulane/Xavier research team to undertake a project.

The project has supported the research work of:

- <u>23 graduate students</u>, including three who were participants in a Tulane/Xavier collaborative mentoring program (3+2= 2) leading to a master's degree in Engineering.
- <u>8 undergraduate students</u>, including 3 Xavier undergraduates (4 worked with a Xavier investigator, 1 worked with a Tulane advisor), 4 Tulane undergraduates and 1 from the University of Virgin Islands who worked on the ONR project with a Tulane advisor. These undergraduates often conducted research with a variety of investigators and thus were exposed to a variety of aspects of the overall project.
- <u>6 SPRITE (undergraduate) students</u> who were chosen out of an applicant pool to follow a mentored graduate-level laboratory research experience and gave a scientific presentation of their research project results to mentors, faculty, and peers at the conclusion of the summer program.

A complete listing of the student names and principal investigators/advisors can be reviewed in Appendix D.

B. Environmental Signals and Sensors

- Three different monoclonal antibodies have been developed for EDTA.
- A new method for producing large quantities of monoclonal antibody in a tissue bioreactor has been developed. The genetic stability of several different hybridomas under culture in this device has been determined.
- The prototype of a fully operational submersible sensor was delivered by Sapidyne Instruments Inc.
- Role for specific signaling pathways identified including the mitogen-activated protein kinase pathway (MAPK) functioning through AP-1 mediated transcription as a critical component of the estrogen mediated cell survival signaling pathway.
- Transcriptional coactivator CBP and P300 represent targets of organochlorine/p38-MAPK mediated phosphorylation.
- Ability of CBP/p300 to control transcriptional activation by a number of transcription factors (CREB, AP-1, HIF ER etc.) further strengthens hypothesis that environmental signaling to gene expression occurs through a common transcriptional target.
- Both the use of peptides that mimic sperm surface proteins as well as the use of UV irradiated sperm can be used to activate *E. coqui* eggs while bypassing normal fertilization.
- Methods have been developed for spermiation, sperm cyropreservation and sperm irradiation, as well as egg irradiation using gamma radiation to inactivate the female pronucleus.
- Long-term frozen sperm from *E. coqui* remains competent to initiate nuclear decondensation in cytoplasmic extracts prepared from *E. coqui* eggs.
- Long-term frozen sperm from these frogs is largely undamaged, capable of initiating development, and that the initial steps at least are largely species independent.
- Two genomic fragments that frequently undergo methylation changes in leiomyoma identified and cloned
- There is a decreased expression of Wnt7a in uterine leiomyoma as compared to that in the adjacent myometrium.
- Imbalance in expression of ER-α and ER-β may contribute to the pathogenesis of uterine leiomyoma in comparing the expression of estrogen receptor ER-α and ER-β mRNA between uterine leiomyoma and adjacent normal myometrium

C. Ecosystem Monitoring and Assessment

• The major pathways for terrestrial carbon from the Mississippi River have been determined for two seasonal time frames in 2000 (Spring-high flow; Fall low flow).

- Two major field experiments have resulted in a large volume of data collected to begin the process of achieving a coordinated understanding of carbon pathways in the study area.
- Web page (<u>www.lumcon.edu/mirir</u>) was maintained to report these major findings to the public, share data with other researchers, and enhance data sharing.
- Partnership with NAVOCEANO has been maintained with linkages and assistance in research development in areas of modeling and field testing.
- Collaboration has been maintained with Woods Hole Oceanographic Institute to
 ensure that CBR biosensor will be compatible with their REMUS AUV, so this can
 be used as a platform for one of the CBR antibody-based biosensors (under
 development by Drs. Diane and Robert Blake as discussed in Environmental
 Signals and Sensors).
- Website (www.leag.org) was established where visualization will be displayed.
- A code based on the LBM to study the sedimentation and re-suspension behavior of single particles and groups of particles was completed.
- Hydrodynamic lift forces exerted by a suspension of particles on a stationary particle are sufficient to cause the re-suspension of the latter without any interparticle collisions.
- Sedimentary particles strongly interact and form dynamic clusters.
- Characteristics of the sedimentation of the clusters are totally different from those of single particles.
- All COAMPS components have been successfully installed on Linux system
- Baseline simulation developed using COAMPS and performance were validated with two studies on western continental US
- Humic acids were determined to facilitate colloid-facilitated transport of contaminants in soil, whereas calcium ion has a profoundly hindering effect against such transport, thus countering the action of humic acids.
- The presence of aluminum ions greatly increased the attachment of iron oxide colloidal particles whether in the absence or in the presence of humic acids
- Presence of aluminum ions is significant in the colloid-facilitated transport and mobilization of contaminants in subsurface environments, where humic substances have previously been known to enhance transport while polyvalent metal ions are noted as promoters of particle aggregation in suspensions and slurries.
- Sediment storage in the lower Mississippi takes the form of two distinct mechanisms.
- First mechanism is found in the lowermost river and is associated with upriver penetration of the salt wedge from the Gulf in periods of low river discharge.
- Second is a freshwater mechanism mapped upriver that is formed as a function of increasing bottom frictional bottom stress on the flow in shallow regions leading to

an overall increase in flow velocity and the ability to transport sediments in suspension.

- No significant change in roughness exponents has been observed for measured systems of gap/particle diameter ratios up to slightly greater than 2.
- In these same systems there was no significant change in the crossover length scale between the two observed roughness exponents of sedimenting particles.
- There are some signs of continuous change with the length scale L, thus suggesting that scaling may not be valid and that alternate methods of analysis should be explored.
- Interfaces may undergo some local shifts while sedimentation is taking place, originating from shifts in beads located deep in the surface.
- Similarity in variability patterns of surface dissolved concentrations of trace elements in Mississippi River have been identified for each season
- Trace metal contents of particulate matter in surface water decreases from the river to the sea in general.
- Mercury isotope ratios in four cores from lower river wetlands with similar variations over time indicates comparable sources of pollution emissions
- The *RV Eugenie* was obtained by CBR in April 2000 through a grant from the Coypu Foundation.
- As a floating laboratory and observatory, the *RV Eugenie* provided support for ONR researchers collecting data through water column and grab sampling, seismic mapping on the Mississippi River.
- ONR funded investigators who utilized the RV Eugenie include Drs. Brent McKee, Tom Bianchi, Franco Marcantonio and Mead Allison from Tulane University Departments of Geology and Ecological and Evolutionary Biology.
- Occurrence and fate of pharmaceuticals and personal care products (PPCP) and endocrine disrupting chemicals as chlorinated byproducts in local waste and drinking waters were identified.
- Analytical methodology was established for low levels of contaminants for application to ONR/DOD biological agent monitoring.

APPENDIX A.

INVESTIGATOR RESEARCH REPORTS

Computer Operations Core

Principal Investigator:

John Vassilopoulos, MS

Director

Computer Operations

Center Bioenvironmental Research

Reporting Period:

May 2001 – April 2002

Primary Objectives of Research Activities

To provide end-user technical direction for data warehousing, networking, securing storage for project-related data, and to coordinate with research support efforts for all CBR core projects.

Progress Made to Achieve these Objectives

Hardware and software acquisitions. Implementation of networking protocols. Web-based information exchange. Data warehousing and safekeeping.

Major Accomplishments

- Established and maintained the IT infrastructure necessary to accommodate all project requirements for analysis and information exchange.
- Completed collaborative efforts in establishing multi-platform wireless connectivity.

Publications, Manuscripts, Abstracts

N/A

Presentations

N/A

Intellectual Development

N/A

Useable Technologies

N/A

Environmental Informatics Core (including Historical Bathymetry of the Lower Mississippi River, conducted under the aegis of Environmental Informatics)

Principal Investigator: Douglas J. Meffert, PhD., Deputy Director

Center for Bioenvironmental Research at Tulane and Xavier Universities

Co-Investigators: Richard Campanella, Assistant Director

Environmental Analysis & GIS/Remote Sensing Specialist

Center for Bioenvironmental Research

Reporting Period: May 2001 – April 2002

Primary Objectives of Research Project

The objectives of Environmental Informatics were to provide geographical analyses, principally through the application of Geographic Information Systems (GIS) technology, to the various research activities progressing under the Integrated Bioenvironmental Hazards Research Program. This work was conducted by personnel in the Environmental Informatics Lab (EIL), the "geography arm" of the CBR. The EIL provides the CBR, its affiliated researchers, its funding agencies, and the general public with a broad range of technical and analytical support in the areas of spatial analysis, GIS, remote sensing, cartography, and database management. Specific ONR research focused on three areas: (1) a detailed and ongoing analysis of the historical bathymetry of the lower Mississippi River during the 20th century (in collaboration with Dr. Bernard Coakley and Dr. Mead Allison); (2) research on the habitat characteristics of the *Aedes aegypti* and *Culiseta melanura* mosquitoes (in collaboration with Dr. Dawn Wesson), and (3) mapping and cartographic production work in relation to Dr. Val Wilson's uterine fibroid research and CBR's efforts toward the development of the National Center for the Mississippi River. Additionally, the EIL is assembling a database of GIS datasets relevant to the lower Mississippi and Gulf of Mexico estuary.

Progress Made to Achieve these Objectives

Substantial progress has been made toward all objectives. Important and hitherto unrecognized patterns in the changing bathymetry of the lower Mississippi River were measured and documented. Field research and statistical analysis of habitat patterns of *Aedes aegypti* and *Culiseta melanura* were carried out. Numerous maps of uterine fibroid occurrence at local, regional, and national levels were produced, as were cartographic products relating to the National Center for the Mississippi River. Finally, a large database of GIS and remote sensing datasets of southeastern Louisiana has been amassed.

Major Accomplishments

Discovery of significant patterns of aggradation and erosion in the lower Mississippi River: Analysis of average depth change per mile from 1921-1992 shows that the lower river has been aggrading above New Orleans and eroding below New Orleans at a rate of roughly one foot per 10 river miles, with most change occurring in the 1921-1948 period. These trends are more pronounced when the change of the deepest point is analyzed per river mile. The data also show that lateral channel changes above New Orleans varied to a slightly greater degree than below New Orleans, which supports the bathymetric observations. Verification of these trends with 19th century datasets is the next step. If verified, we will propose hypotheses to explain two empirical observations: why has the river aggraded and eroded in this manner, and why did this trend diminish later in the century.

Establishment of a two-stage field experiment for mosquito habitat characterization: Stage 1 consisted of a seven-point stratified experimental design in the Northlake Nature Center and Fountainebleu State Park near Mandeville, Louisiana; stage 2 comprises a more aggressive 15-point grid in a systematic design focused on a GIS-selected area of high topographic and ecological diversity in Northlake Nature Center. Using GPS, measuring tape, and machetes, we established the grid in May 2001. Four mosquito traps (fiber resting pots) as well as a rain gauge and weather data logger are mounted at each site. A team of 2-3 people visit every site each week and collect mosquitoes from each trap using an aspirator (essentially a blower through into reverse, to such mosquitoes out of the resting boxes and into special containers. Specimens, often numbering in the hundreds, are then analyzed in Dr. Wesson's lab by graduate students. For the initial spatial analysis, conducted by the EIL in the Fall of 2001 and later updated, a series of statistically significant correlations between species occurrence and habitat types were found.

Publications

John J. Galler, Thomas S. Bianchi, Mead A. Allison, Richard Campanella & Laura Wysocki. In preparation: *Biogeochemical Implications of Levee Confinement in the Lowermost Mississippi River* Journals considered for submission include *EOS Transactions*, American Geophysical Union.

Presentations

Patterns of Bathymetric Change in the Lower Mississippi River, 1921-1992. Presented by Richard Campanella in increments at the Louisiana Remote Sensing-Geographic Information Systems Conferences, April 2001 (poster session) and April 2002 (oral presentation), in Baton Rouge, Louisiana, and the American Society for Limnology and Oceanography Conference, Spring 2001 (poster session), in Albuquerque, New Mexico. Research co-authored by Dr. Bernard Coakley and Dr. Mead Allison.

Habitat Characteristics of Potential Arbovirus: Field Observations in St. Tammany Parish, Louisiana. Presented by Richard Campanella at the Louisiana Mosquito Control Association Conference, October 2001, in Lake Charles, Louisiana. Research co-authored by Dr. Dawn Wesson and others.

The Environmental informatics Lab (EIL) On-line Mapping System. Presented in increments by Stephanie L. Smith at the 18th Annual Remote Sensing and GIS Workshop, Baton Rouge, La. April 9-11, 2002, and GeoSpatial World 2002, Atlanta, Georgia, June 10-12, 2002.

Intellectual Development: N/A

Useable Environmental Technologies: N/A

Education Core

SPRITE: The <u>SummerP_ipeline Research Initiative</u>: the <u>Tulane Experience</u>
A Mentored Introduction to Programs of Study for Graduate Research

Principal Investigator: Valerie Petit Wilson, Ph.D. formerly

Clinical Associate Professor, Environmental Health Sciences

Deputy Director, Center for Bioenvironmental Research

at Tulane and Xavier Universities

Co – Investigators: Charles E. Allen, III, MSPH

Education and Outreach Coordinator Center for Bioenvironmental Research

Dana M. Greene-McDowelle, Ph.D.

Assistant Professor, Biology Xavier University of Louisiana

Reporting Period: May 2001 - April 2002

Primary Objectives of Research Activities

The Summer Pipeline Research Initiative: the Tulane Experience (SPRITE) is an educational initiative that the Center for Bioenvironmental Research (CBR) coordinates between Tulane University's (TU) Molecular and Cellular Biology (MCB) Graduate Program and Xavier University's (XU) Office of Sponsored Programs. The goal of SPRITE is to increase the number of minorities at the graduate level in the bioenvironmental and biomedical sciences by:

- Providing Xavier undergraduate students with a quality bench research experience in an MCB laboratory under the guidance of an established researcher; and
- Exposing these students to graduate life and the MCB program of Tulane University.

The program focuses on two unique resources: Tulane's successful biomedical graduate program and Xavier University's outstanding pool of science majors. The intent of the program is to provide a mentored introduction to Tulane's excellent programs of graduate study with a successful research experience partially supported by ONR.

In addition to the research, students also participate in weekly seminars and roundtable discussions led by the SPRITE program staff. These seminars cover areas such as research at the frontiers of science, financial aid and career opportunities. In addition, students are provided with exposure to scientific ethics using the National Academy of Sciences Text "On Being A Scientist" in addition to a lecture/discussion on biomedical ethics. Other sessions include forums on the presentation of a research seminar and training on graphics and presentation skills.

The culminating event for the summer activities is a research symposium in which student interns present their work to the assembled faculty, students, laboratory colleagues and staff. Subsequently, in the following fall, students have access to SPRITE staff for assistance in applying to graduate and professional schools for the appropriate academic year.

Progress Made to Achieve These Objectives

- For summer 2001, program coordinators developed application packets and disseminated information to eligible sophomores, juniors, and seniors at Xavier University. Presentations were made to faculty and students at Tulane and Xavier Universities to recruit both mentors and research interns.
- In summer 2001, six students were competitively selected from a pool of 26 applicants for the 10-week summer program. An additional two students were involved in the program. The first was from the University of the Virgin Islands was conducting research in the laboratory of a CBR faculty. The second was from Xavier University and was involved in the school's Ronald McNair Scholars Program. Both students had their stipends and needed only to have a structured research experience.
- The summer 2001 program involved faculty research mentors from the following disciplines: Medicine/Nutrition, Microbiology/Immunology, Ophthalmology, Pathology and Tropical Medicine.
- Two SPRITE interns from the 2001 summer program attended the 11th Annual Student Technical Conference of the Science and Engineering Alliance in collaboration with the 100th Centennial Celebration of the National Institute of Standards and Technology which was held October 11-13, 2001 in Gaithersburg, Maryland. This event was not a competition.
- Ashte' Collins presented his project entitled, Cell-Signaling Proteins P53 and AKT as Prognostic Markers in Lung Adenocarcinoma conducted under the guidance of Sanda Clejan, PhD, Professor, Department of Pathology, Tulane University Health Sciences Center (TUHSC).
- William Cody presented his project entitled, Serotyping of Pseudomonas aeruginosa, conducted under the guidance of Michael Schurr, PhD, Assistant Professor, Department of Microbiology/Immunology, TUHSC.
- Activities by the SPRITE coordinators in fall 2001 included assistance with student applications to graduate school and other professional schools.
- In three years, the SPRITE program has become a major pipeline of minority students to Tulane graduate and health professional schools. Of the six students who completed the 2001 summer program, two were accepted to medical schools (one to Tulane, and the other to the University of Chicago, Medical School), one was accepted to Cornell University Graduate School, another graduated and has taken a year off from academics, and the remaining two are finishing their final two undergraduate years. One of these students has been accepted to Tulane Medical School through an early admissions program.
- Selection for SPRITE interns for the summer 2002 program was equally competitive; 25 applications were received for the 6 positions. Funding for this set of interns was shared between ONR (2001 funding) and Xavier University's Model Institutions of Excellence (NSF project grant).
- The success of SPRITE provides a national model for pipeline partnerships between research institutions and historically black colleges/universities.

Publications

N/A

Presentations

By Students – First, the 2001 interns presented at the CBR's own research symposium which was held on Friday, August 3, 2001 at the CBR. Second, the interns presented at the 11th Annual Student Technical Conference of the Science and Engineering Alliance in collaboration with the 100th Centennial Celebration of the National Institute of Standards and Technology, October 11-13, 2001(see above for description).

Intellectual Development

- 1. Student Name: Juliet Aregbesola
- 2. Funding Period: May through August 2001
- 3. **Duties and Responsibilities**: Student was responsible for conducting research on the project entitled "The Role of Interferons in the Inhibition of Herpes Simplex Virus Immediate Early Promoters"
- 4. Research Advisor: Dr. William Halford, Microbiology and Immunology, TUHSC
- 1. Student Name: William Cody
- 2. Funding Period: May through August 2001
- 3. **Duties and Responsibilities**: Student was responsible for conducting research on the project entitled "Serotyping of Pseudomonas aeruginosa"
- 4. Research Advisor: Dr. Michael Schurr, Microbiology and Immunology, TUHSC
- 1. Student Name: Ashte' Collins
- 2. Funding Period: May through August 2001
- 3. Duties and Responsibilities: Student was responsible for conducting research on the project entitled "Cell-Signaling Proteins P53 and AKT as Prognostic Markers in Lung Adenocarcinoma"
- 4. Research Advisors: Drs. Sanda Clejan and Odile David, Pathology, TUHSC
- 1. Student Name: Shakia Davis-Moliere
- 2. Funding Period: May through August 2001
- 3. **Duties and Responsibilities**: Student was responsible for conducting research on the project entitled "Diet differences in Non-Hispanic White and Black Males as Related to Heart Disease"
- 4. Research Advisor: Dr. Karen Friday, Medicine-Endocrinology, TUHSC
- 1. Student Name: Lena Gamble
- 2. Funding Period: May through August 2001
- 3. **Duties and Responsibilities**: Student was responsible for conducting research on the project entitled "Peripheral Blood Mononuclear Cells as a Possible Marker for the Detection of the Expression of CYP 2C9 Gene"
- 4. Research Advisor: Drs. Sanda Clejan and Shanker Japa, Pathology, TUHSC
- 1. Student Name: Alayna Stepter
- 2. Funding Period: May 2000 August 2001
- 3. **Duties and Responsibilities**: Student was responsible for conducting research on the project entitled "The Survival of Water-borne Viruses: Modeling the MS2 Bacteriophage to test its survival through a new water purification process"
- 4. Research Advisor: Dr. Scott Michael, Tropical Medicine, TUHSC

Useable Technologies

N/A

Foundations of a five year Xavier Tulane Program leading to a Masters degree in Engineering

Principal Investigator:

Elia Eschenazi, PhD.

Associate Dean, Physics and Engineering

Xavier University

Co-Investigator:

Stathis Michaelides, PhD.

Associate Dean, Engineering

Tulane University

Reporting period:

May 2001 – April 2002

Primary Objectives of Research Project

This project proposes the establishment of the foundations of a five year program leading to a master degree in engineering from Tulane University and BS in Physics / Chemistry at Xavier University. This program will allow the students in the Departments of Physics/Engineering at Xavier to complete the requirements for both the bachelor's degree and the master's degree in the School of Engineering at Tulane within five years from their matriculation. Summer research work will be required for the timely completion of the master's thesis.

The purpose is to continue consolidating and enhancing the pipeline producing more African American masters' and PhD's. In the development of the program students will be involved for two summers of their 3rd and 4th year in research projects which will lead to their master thesis. This not only will create a substantial basis for the successful development of the program but it will also foster collaboration of the Universities in research areas relevant to the Office of Naval Research.

Progress Made to Achieve these Objectives

Three students were initially accepted in the program beginning their 4th year at Tulane. Two students in mechanical engineering, Lorenzo Craig and Melodie Wyche and one student in chemical engineering, Monique Gibbs.

Monique Gibbs, graduated with a Masters in Chemical Engineering from Tulane University and a BS in Chemistry from Xavier University in May 2003.

Lorenzo Craig is completing his thesis which he will defend before the end of the summer.

Melodie Wyche is expected to complete her course work and the masters' thesis by the end of the Fall '03.

Three additional students joined the program: DeMarkius Erwin (Mechanical Engineering), Brandi Tregre (Biomedical Engineering) and Folami Morris (Mechanical Engineering). Two more students applied. Their applications are presently being reviewed.

The 3+2 Xavier/Tulane program leading to masters in engineering has been institutionalized. An articulation agreement has been signed by the Presidents of both Universities.

Initially the program included only chemical, mechanical and environmental engineering. Presently it includes other engineering fields such as biomedical.

The team effort of Xavier/Tulane faculty in mentoring the students and the strong collaboration in the various research projects has consolidated the training/research environment and the partnership which are fundamental ingredients for the success of the program.

The program has also enhanced research capability and competitiveness at Xavier Universities by increasing scholarly publications and attracting more research funding.

Intellectual Development

- 1. Student Names: Lorenzo Craig, Melodie Wyche, Monique Gibbs
- 2. **Period of funding:** May 2001 April 2002
- 3. **Duties and Responsibilities:** Theses and projects assigned Lorenzo Craig: "Influence of suspended particles on the hydrodynamic force acting on a stationary particle" (expected graduation August '03). A paper on this study is in preparation and it will submitted in the fall.

Monique Gibbs: "Adsorption of Humic Acid in the Presence of Al3+ and its Effects on Colloid-Facilitated Transport". (graduated in May '03). A paper on this study is in preparation and it will submitted by the end of the summer.

Melodie Wyche: "Influence of Basset force on the dynamics of particles in unsteady, periodically driven flows" (expected graduation December '03)

Symposium on the Environment & Hormones (e.hormone), 2001

Principal Investigator:

John A. McLachlan, PhD

Weatherhead Distinguished Professor and Director

Center for Bioenvironmental Research

Reporting Period:

May 2001 – April 2002

Primary Objectives of Research Project

One of the central themes of the CBR's Integrated Bioenvironmental Hazards Research Program is understanding how bioenvironmental contaminants can impact the health of humans and wildlife and their progeny through disruption of the endocrine system. Understanding the many issues surrounding environmental endocrine disruption, or environmental signaling (eg. contaminants and pollutants) and its effects on human and ecosystem health requires a synthesis of disciplines ranging from molecular biology to systemic population biology. This becomes a daunting task since the scientific terminology and methodology, the meetings attended, and literature read by researchers does not in many cases overlap. The CBR responded to the need for a scientific forum for information exchange and collegial interaction for scientists involved in environmental signaling research by hosting the first international Symposium on the Environment and Hormones (e.hormone) in October 1999. e.hormone has become an annual event.

The goal of this symposium series is to bring together innovative thinkers, cutting edge researchers, and key decision makers to critically evaluate current research on environmental signaling and contribute to the future of this new field. The third annual e.hormone symposium took place in New Orleans October 18-20, 2001. It was a truly multidisciplinary, multinational event. Presentation topics ranged from human to ecosystem health, from basic research to population studies. It was an active meeting with lively discussions of the hottest issues; formal and informal networking opportunities were built into the schedule of e.hormone symposium activities.

e.hormone 2001 was host to 39 speakers (10 international) and 210 participants (37 international). Ecologists, chemists, endocrinologists, toxicologists, zoologists, engineers, philosophers, undergraduate science faculty, high school teachers, policy makers, and media from the United States, Japan, Europe, and Latin America came together to analyze the latest findings on environmental signaling that are the basis of endocrine disruption. Explorations remained at the cutting edge in research and policy.

Educational Objectives:

After attending this continuing education activity, the participant should have been able to:

- Interpret cutting edge research and techniques related to environmental signaling and apply current knowledge to future research or decision-making.
- Identify research interests and colleagues in the field of environmental signaling that will foster future collaboration or information exchange
- Understand philosophical approaches, concepts, frameworks, and policy implications related to endocrine disrupting chemicals and environmental signaling.

Progress Made to Achieve these Objectives

e.hormone has become the focal point for all those who are interested in the field of environmental signaling. Sessions were held at the CBR conference facility in the Health and Environmental Research Building in downtown New Orleans. The symposium format included scientific presentations grouped around conceptual themes. Participants felt the organization of the program, with several talks on a topic (e.g. prostate development, breast cancer, evolution) was a welcome antidote to the usual dizzying fragmentation of subjects at conferences. The organizing committee, cognizant of the latest in literature and findings, selected presenters who are conducting cutting edge research across a variety of disciplines and represent diversity in race/ethnicity, gender, geographic, and senior/junior research status. This was an additional strength of the carefully-constructed program; welcoming fresh voices to present interesting new work.

e.hormone 2001 featured exciting presentations and information exchanged in a wonderful collegial atmosphere. The theme of signaling across organisms and the susceptibility of numerous systems to the deleterious effects of environmental hormones was also an effective organizing principle. In general the topic sessions, representation from European and Asian scientists, and the caliber of the talks resulted in a highly productive and enjoyable meeting for all participants.

Examples of ONR-related research topics and themes at e.hormone 2001 include the following presentations: Ann Cheek (Southeastern Louisiana University) Introduction and Overview; Shinsuke Tanabe (Ehime University, Japan) Global Contamination and Toxic Effects of Bioaccumulative Endocrine Disrupters in Humans and Wildlife;" Douglas Meffert (CBR) and Angela Preimesberger (Environmental Toxicology Consultant, Minneapolis) Human & Ecological Species Integration for Functionally-based Assessments: a Bisphenol-A Case Study; Tyrone Hayes (University of California, Berkeley) Ecologically Relevant Atrazine Doses Disrupt Sex Differentiation: African Clawed Frog Xenopus Laevis; Carol Meteyer (United States Geological Survey) Encyclopedia of Frog Malformations in the Wild; Mary Beth Martin (Georgetown University) Cadmium: an Endocrine Disrupter?; and Vance Trudeau (University of Ottawa, Canada) Expression Profiling Estrogen and Xenoestrogen Action in the Nonmammalian Vertebrate Brain.

Major Accomplishments

Throughout its three-year history, the e.hormone symposium has resulted in the creation of an extensive global network. Major accomplishments include:

- Increased participation attendance rose from 136 in 2000 to 210 in 2001
- Symposium continuity many returning attendees from 2000 as well as new attendees in 2001
- Comprehensive poster session for junior investigators 26 posters featured in 1999, 39 posters in 2000, and 64 posters in 2001
- A global approach to science -7 of the 26 speakers in 2000 were international, and there were 19 international registrants; in 2001, 10 of the 39 speakers were international and there were 37 international registrants
- Growing research relationships with Japanese colleagues 14 attended in 2000, 18 in 2001

- Maintenance of the "spin-off" e.hormone website as a hub of scientific and media information connecting research colleagues throughout the year
- Creation of a mentoring/networking forum for junior investigators 50 students and postdoctoral fellows registered

Publications, Manuscripts, Abstracts

Each of the past three symposia has been reported on the web, and its scholarship recognized in publications such as *Science News*.

Presentations

e.hormone 2001 sessions:

- I Environmental Factors & Human Sexual Development
- II Sex Reversal: Fish
- III Hormonally Active Agents in the Environment
- IV Mechanisms of Environmental Hormone Action
- V Hormones in Male Reproductive Tract Development
- VI Environmental Estrogen Factors & Breast Disease
- VII Federal Programs in Endocrine Disruption: Priority Needs & Future Directions

Intellectual Development

N/A

Useable Environmental Technologies

While no technologies have resulted directly from the e.hormone workshop series, the CBR deems that interdisciplinary workshops like this one are critical for the creation of scientific collaborative approaches that foster biosensor development. Such biosensors harness the power of "environmental signaling," and lead us to further exploration and development of numerous near real-time monitoring technologies for the ONR, in particular, and the DOD, in general.

Antibody-Based Biosensors for Autonomous Underwater Vehicles

Principal Investigator: Diane A. Blake, PhD.

Associate Professor

Department of Ophthalmology

Tulane University Health Sciences Center

Co-Investigators: George C. Flowers, PhD.

Associate Professor and Chair

Department of Geology

Tulane University

Robert C. Blake II, PhD. Professor and Interim Chair

Department of Basic Pharmaceutical Sciences

Xavier University of Louisiana

Reporting Period:

May 2001 - October 2001

Primary Objectives of Research Project

The goal of this project is to develop a biosensor that will permit the rapid, automated identification and quantification of EDTA in river water. A set of high-affinity, highly selective binding reagents (antibodies) will be used to develop an immunosensor for EDTA that can operate in an autonomous underwater vehicle (AUV). The specific aims for this project are to 1) isolate and characterize monoclonal antibodies that bind tightly and specifically to EDTA; 2) test antibody activity in the environmental sample matrix (Mississippi River water); and 3) direct the progress of Sapidyne Instruments to construct a prototype immunosensor that can be deployed in an AUV.

Major Accomplishments

- Three different monoclonal antibodies have been developed for EDTA. Two antibodies (1B11 and 2D42) show unusual cooperative binding behavior. A third antibody (4B33, which binds to non-cooperatively EDTA with nanomolar affinity) will be used for subsequent sensor development. In addition, 15 different, well-characterized monoclonal antibodies are available for these immunosensor studies.
- A new method for producing large quantities of monoclonal antibody in a tissue bioreactor has been developed. The genetic stability of several different hybridomas under culture in this device has been determined.
- The prototype of a fully operational submersible sensor was delivered by Sapidyne Instruments Inc. The fluidics of this system were tested and were fully functional. The optical/sensing unit was poorly designed and will require some modifications before it operates with optimal sensitivity.

Publications, Manuscripts, Abstracts

- D.J. Meffert, D.A. Blake, R.C. Blake II, and R.G. Rey (2000) "Biosensor Development for Autonomous Real-Time Monitoring of Environmental Toxicants", Proceedings of Autonomous Undersea Systems Institute: Sensors and Sensing Technology for Autonomous Ocean Systems Workshop, Waikoloa, HI.
- D.A. Blake, R. M. Jones, R.C. Blake II, A.R. Pavlov, I.A. Darwish, and H. Yu (2001) "Antibody-based sensors for heavy metal ions", *Biosensors and Bioelectronics*, **16**:799-809.
- D.J. Meffert, D.A. Blake, R.C. Blake II, and R.G. Rey (2001) 'Antibody, cell-based, and transgenic fish biosensors for autonomous monitoring of environmental toxicants", Proceedings of Autonomous Undersea Systems Institute: Sensors and Sensing Technology for Autonomous Ocean Systems Workshop, Newport, RI.
- R.M. Jones, H. Yu, J.B. Delehanty, and D.A. Blake (2002) "Monoclonal antibodies that recognize minimal differences in the 3-dimensional structures of metal-chelate complexes", *Bioconj. Chem.*, **13**: 408-415.
- J.B. Delehanty, R.M. Jones, T.C. Bishop and D.A. Blake (2003) "Identification of important residues in metal-chelate recognition by monoclonal antibodies", *J. Biol. Chem.*, submitted.

Presentations

- D.A. Blake, R.C. Blake II, Haini Yu, and R.M. Jones (2001) "Immunosensors: An emerging technology", Biosensors Workshop, Division of Small Chemical Businesses, 221st ACS National Meeting, San Diego, CA, April 1-5.
- C.J. Phillip, R.M. Jones, S.C. Lorbach, and D.A. Blake (2001) "Comparison of a one-step and two-step immunoassay for cadmium in water samples", Annual Biomedical Research Conference for Minority Students, Orlando FL, Oct. 31-Nov. 3.
- A.M. Kriegel, H. Yu, J.B. Delehanty, R.M. Jones, and D.A. Blake (2002) "Antibody-based sensors for metals Present studies and future directions" Board of Regents' NSF/LA EpSCOR 2002 Conference, Baton Rouge, LA, April 10-11.

Intellectual Development

- 1. **Student Names:** James B. Delehanty, Alison M. Kriegel (graduate students in the Interdisciplinary Graduate Program in Molecular and Cellular Biology) and Cornel J. Phillip, a summer minority undergraduate researcher from University of the Virgin Islands.
- 2. **Period of funding:**

James B. Delehanty, 5/29/00-6/30/01(received Ph.D. 6/25/01). Alison M. Kriegel, April 2001-April 2002.

Cornel J. Phillip's stipend and expenses from 6/01-8/01 were funded through an NIH-funded MBRS-RISE program at the University of the Virgin Islands.

3. Brief description of duties and responsibilities:

Delehanty cloned, sequenced, and expressed a recombinant antibody that recognized chelated complexes of Pb.

Kriegel prepared large quantities of monoclonal antibodies for further development work. She also prepared proteolytic fragments of several monoclonal antibodies, and determined the binding characteristics of anti-chelate monoclonal antibodies.

Phillip learned how to perform immunoassays for metal ions and compared assay performance in different environmental matrices.

Useable Environmental Technologies:

- 1. **Title of technology product**: Immunosensor for deployment in AUV; Recombinant antibodies for environmental analysis
- 2. **Description of technology product**: This antibody-based biosensor will be able to automatically collect and analyze 5 separate samples after installation in an autonomous underwater vehicle or immobilized buoy (EARS);
- 3. **Utility/benefit/ROI/payoff of technology product**: A self-contained, automated immunosensor will have the capability to detect very low concentrations of environmental contaminants and/or chemical and biological weapons in surface waters.
- 4. **Timeline**: An assay that detects nanomolar levels of EDTA, the first analyte to be developed for this instrument, has been established. Transfer of the assay to the immunosensor will begin when Sapidyne has corrected the defects in the optical components of the instrument.
- 5. Partners: Sapidyne Instruments (Boise, ID) is constructing the immunosensor and our laboratory is working closely with them to coordinate the development of biological reagents with the development of the instrument. The Blake laboratories also have strong ties with Dr. Fran Ligler's laboratory at the Naval Research Laboratory in Washington D.C. James Delehanty, who recently received his Ph.D. in Diane Blake's laboratory, is now an NRC fellow in Dr. Ligler's laboratory.

Effects of Estrogens and Endocrine disrupters on Suppression of Apoptosis in normal and neoplastic Breast epithelial cells

Principal Investigator: Matthew E. Burow, Ph.D.

Assistant Professor

Center for Bioenvironmental Research Department of Medicine and Surgery

Tulane University

Co-Investigator(s): John A. McLachlan, Ph.D.

Professor and Director

Center for Bioenvironmental Research

Department of Pharmacology

Tulane University

Reporting Period: May 2001 – April 2002

Primary Objectives of Research Activities

The major goals of this project where to 1) develop technologies and methods to identify relevant organochlorine chemicals and dietary flavonoids that exert effects on estrogen responsive tissues and cell survival pathways, and 2) identify mechanism by which selected environmental agents would subvert the estrogen and cell survival signaling pathways thereby leading to potential developmental defects and or disease states (i.e cancer).

Progress Made to Achieve these Objectives

During the funded period we have identified a role for specific signaling pathways including the mitogenactivated protein kinase pathway (MAPK) functioning through AP-1 mediated transcription as a critical component of the estrogen mediated cell survival signaling pathway. The ability of estrogenic chemicals (estradiol, DES, DDT) to exert effects on cell survival pathways of breast carcinoma cells required an intact ERK-MAPK-pathway (1,4). In contrast anti-estrogens such as tamoxifen or ICI 182,780 as well as dietary flavonoid anti-estrogens exert negative effects on cell survival and ER activity through the JNK and p38 pathways (2-4,7). This understanding of the basic mechanisms of cell survival signaling through ER, AP-1 and MAPKs allowed us to develop in vivo screening technologies for AP-1 activating chemicals using stably transfected human endometrial and human embryonic kidney cell lines. These cell systems have allowed us to examine the ability of selected chemicals to activate AP-1 and related signaling pathway (Fos, Jun, Creb, Elk, Chop) through ER-dependent and independent mechanisms (5,6). The ability of environmental agents to regulate cell signaling and AP-1 pathways in an ER-independent mechanism forces us to expand our search for relevant endocrine disruption chemicals to include those outside the realm of estrogenic compounds. Subsequent to this an additional manuscript has been completed during the 2001-2002 year. This publication (9) now demonstrates the ability of specific organochlorine chemicals to signal to AP-1 mediated gene expression via activation of selective MAPK pathways. In this in press publication we demonstrate a primary role for the p38-MAPK cascade but not Erk, JNK or BMK-1 in regulation of AP-1 by organochlorines. Subsequent studies have now established

that specific organochlorines can activate a number of other signaling pathways including antioxidant response elements, hypoxia-induced factor 9HIF) and CRE. This suggests that common targets exist for the organochlorine/p38-MAPK cascade in the regulation of environmental responsive gene expression. We have identified that the transcriptional coactivator CBP and P300 represent targets of organochlorine/p38-MAPK mediated phosphorylation. The ability of CBP/p300 to control transcriptional activation by a number of transcription factors (CREB, AP-1, HIF ER etc.) further strengthens our hypothesis that environmental signaling to gene expression occurs through a common trancriptional target. We are now focusing our efforts on identifying the mechanisms and role of organochlorine MAPK targeting of coactivators.

Major Accomplishments

- Established role for specific MAPKs (p38) in organochlorine regulation of AP-1-mediated gene expression (publication #9)
- Identified a role for MAPK signaling in conjunction with Bcl-2 expression in estrogen mediated cell survival signaling in breast carcinoma cells. (publication # 4)
- Used relevant estrogen responsive reporter technologies to screen flavonoid phytochemicals for estrogenic and anti-estrogenic activities towards MCF-7 breast carcinoma cells (publications #2,3)
- Identified a role for JNK and p38 MAPKs in signaling by flavonoid phytochemicals in the regulation of ER-mediated gene expression and proliferation of breast carcinoma cells (publication # 2,5,7)
- Identifies those flavonoid phytochemicals that demonstrate the ability to induce apoptosis or programmed cell death in human breast carcinoma cells (publication #8)
- Correlated relative estrogen receptor alpha and beta expression and signaling with apoptotic sensitivity and resistant among breast cancer cell variants (manuscript #1)
- Developed an *in vivo* mammalian cell culture assay to examine environmental relevant organochlorine molecules for estrogen receptor dependent and ER-independent activity toward cell signaling via mitogen-activated protein kinase (MAPK)-mediated activation protein 1 (AP-1) transcription. (publications # 4,5)
- Identified a role for organochlorine pesticides and flavonoid phytochemicals signaling to AP-1 via ER-independent mechanisms (publications #5-7, 9).

Publications, Manuscripts, Abstracts

Burow, M.E., Weldon, C.B., Chiang, T-C., Tang, Y., **Collins-Burow B.M.**, Rolfe, K., Li, S., **McLachlan, J.A.**, Beckman, B.S. Differences in protein kinase C and estrogen receptor α, β expression and signaling correlate with apoptotic sensitivity of MCF-7 breast cancer cell variants. *Int. J. Oncol.* 16: 1179-1187, (2000).

Collins-Burow, B.M., Burow, M.E., Duong, B.N., McLachlan, J.A. Estrogenic and antiestrogenic activities of flavonoid phytochemicals through estrogen receptor binding-dependent and -independent mechanisms. *Nutrition and Cancer.* 38(2), 229-244 (2000).

Burow, M.E., Boue, S.B., **Collins-Burow, B.M.,** Melnik, L.I., Duong, B.N., Li, S.F., Wiese, T., Cleavland, E., **McLachlan J.A.** Phytochemical glyceollins, isolated from soy, mediate anti-hormonal effects through estrogen receptor alpha and beta. *J. Clin. Endocrinol. and Metabolism* **86(4)**, 1750-1758, (2001).

- **Burow, M.E.**, Weldon, C.B., Tang Y., **McLachlan, J.A.**, Beckman, B.S. Oestrogen -mediated suppression of TNF-induced apoptosis in MCF-7 cells: subversion of Bcl-2 by anti-oestrogens. *J. Steroid Biochem. & Mol. Biol.* **78**(5): 409-418, (2001).
- **Frigo, D.E.,** Duong, B.N., Melnik, L.I., Schief, L., Collins-Burow, B.M., Pace, D.K., **McLachlan, J.A.**, **Burow, M.E.** Flavonoid Phytochemicals Regulate Activator Protein-1 Signal Transduction Pathways in Endometrial and Kidney Stable Cell Lines. *Journal of Nutrition* **132(7)**:1848-1853, (2002).
- **Frigo, D.E., Burow, M.E., Mitchell, K.A.,** Chiang, T-C., **McLachlan, J.A**. DDT and its metabolites alter gene expression in human uterine cell lines through ER-independent mechanisms. *Environmental Health Perspectives* **110(12)**:1239-1245, (2002).
- **Burow, M.E., Collins-Burow, B.M., Frigo, D.E.,** Weldon, C.B., Elliot, S., Alam, J., **McLachlan, J.A.** Antiestrogenic activity of flavonoid phytochemicals mediated via c-jun N-terminal protein kinase and p38, Mitogen-activated protein kinase pathways. Isoform specific antagonism of estrogen receptor alpha. In preparation for submission to *Endocrinology*.
- Collins-Burow, B.M., Burow, M.E., Weldon, C.B., McLachlan, J.A. Induction of apoptosis by antiestrogenic phytochemicals in breast carcinoma cells. Manuscript in preparation for submission.
- Frigo, D.E., Tang, Y., Beckman, B.S., Scandurro, A.B., Alam, J., Burow, M.E., McLachlan, J.A. Mechanism of AP-1-mediated gene expression by select organochlorines through the p38 MAPK pathway. *Carcinogenesis* (2004). In press

Presentations

- Frigo, D.E., Burow, M.E., Mitchell, K.A., Elliott, S., McLachlan, J.A. The Effects of DDT and its Metabolites on AP-1 Activity: ER Dependent and Independent Mechanisms. <u>AACR Annual Meeting.</u> <u>March 28th, 2001-New Orleans, LA</u>.
- **Frigo, D.E., Burow, M.E.,** Mitchell, K.A., Elliott, S., **McLachlan, J.A.** The Effects of DDT and its Metabolites on AP-1 Activity: ER Dependent and Independent Mechanisms. 13th annual Tulane Health Sciences Research Days, 2001.
- Frigo, D.E., Burow, M.E., Mitchell, K.A., Elliott, S., McLachlan, J.A. The Effects of DDT and its Metabolites on AP-1 Activity: ER Dependent and Independent Mechanisms. 2001 Environmental Signals and Sensors Center for Disease Control Meeting.
- **Frigo, D.E., Burow, M.E.,** Mitchell, K.A., Elliott, S., **McLachlan, J.A.** The Effects of DDT and its Metabolites on AP-1 Activity: ER Dependent and Independent Mechanisms. Gordon Research Conference, Hormonal Carcinogenesis. July 10th, 2001-Meriden, NH
- Frigo, D.E., Burow, M.E., Mitchell, K.A., Chiang, T.C., McLachlan, J.A. The Effects of DDT and its Metabolites on AP-1 Activity: Mechanisms of Environmental Signaling. E.Hormone 2001 Conference. At Tulane University, Oct. 18th, 2001-New Orleans, LA.

Frigo, D.E., Burow, M.E., Mitchell, K.A., Chiang, T.C., **McLachlan, J.A.** . The effects of DDT and its metabolites on AP-1 activity: mechanisms of environmental signaling (<u>Abstract 5126</u>). Proceedings of the American Association for Cancer Research, 2002

Daniel E. Frigo, Matthew E. Burow, Jawed Alam, and John A. McLachlan. Endocrine Disruptors Potentiate Coactivators: The role of MAPKs. E.Hormone 2002 Conference, at Tulane University. Oct. 17th, 2002-New Orleans, LA

Frigo, D.E., Burow, M.E., Mitchell, K.A., Chiang, T-C., Alam, J., **McLachlan, J.A.** Endocrine disruptors potentate coactivators: The role of MAPKs. 14th Annual Tulane Health Sciences Research Days, 2002.

Burow, M.E., Melnik, L.I., Elliot, S.E., Collins-Burow, B.M., Alam, J., Hill, S.M., Beckman, B.S., **McLachlan, J.A.** G-Protein Regulation of Nuclear/Steroid Hormone Receptor Activation Through p160 Coactivator Targeting. Keystone Symposia, Nuclear Receptor Superfamily 2002.

Frigo, D.E., Burow, M.E., Mitchell, K.A., Chiang, T-C., McLachlan, J.A. Endocrine disruptors potentiate coactivators: The role of MAPKs. Keystone Symposia, Nuclear Receptor Superfamily 2002.

Intellectual Development

- 1. Student(s) Name: Frigo, Daniel E. and Vigh, Katinka
- 2. Period of funding: Frigo (Graduate Student 2001-2002); Vigh (Undergrad Thesis project 2001-2002)
- 3. Duties and Responsibilities: Frigo: Ph.D. completed December 2003)
 Dissertation title: "DDT AND ITS METABOLITES SIGNAL NUCLEAR TRANSCRIPTIONAL REGULATORS THROUGH A NON-ER-MEDIATED MECHANISM: A MODEL OF ENVIRONMENTAL STRESS SIGNALING"
 Present: Post-doctoral fellow Duke University

Vigh: Tulane University (B.A. received 2002) Present: Graduate Student University of Chicago

Useable Technologies

During the funding period in vivo cell culture models have been established for the examination of cell signaling pathways activation by relevant environmental contaminants. The cell systems can be utilized to screen extracts, mixtures of individual chemicals for activity on known cellular events involved in environmental toxicant responses. This type of screening would provide information as to potential deleterious effects of certain environmental chemicals or methodologies to classify these chemicals based upon unique cell-signaling profiles.

Remediation: Resurrection of an Extinct Species of Frog from Puerto Rico

Principal Investigator:

Scott F. Michael, Ph.D.

Department of Tropical Medicine

Tulane University School of Public Health and Tropical Medicine

Reporting Period:

May 2001 – April 2002

Primary Objectives of Research Activities

- 1) Determine if frozen sperm remain competent to direct development of enucleated eggs from the same species.
- 2) Determine if frozen sperm are able to direct development of enucleated eggs from a closely related species.
- 3) Determine if frozen sperm from an extinct species are able to direct development of enucleated eggs from a closely related extant species.

Progress Made to Achieve these Objectives

An inability to easily initiate egg activation and development in unfertilized eggs has been a major roadblock for this project. To overcome this, large numbers of eggs have been needed for a variety of alternate experimental plans. We were successful in obtaining small numbers of eggs from naturally breeding animals by separating the males and females before fertilization. Larger numbers of eggs could be obtained by artificially induced ovulation, as is done with many other amphibian species. However, injections of human chorionic gonadotropin do not cause ovulation in E. coqui, as they do in some other species. One of our successes has been to identify a synthetic leutinizing hormone releasing hormone that produces consistent ovulation and egg deposition in E. coqui. This has led to the ability to test a wide variety of egg activation strategies, some of which are candidates for consistent use in this species.

None of the commonly used amphibian egg activation techniques appear to work for E. coqui eggs. However, we have preliminary evidence that both the use of peptides that mimic sperm surface proteins as well as the use of UV irradiated sperm can be used to activate E. coqui eggs while bypassing normal fertilization. During this work we have developed methods for spermiation, sperm cyropreservation and sperm irradiation, as well as egg irradiation using gamma radiation to inactivate the female pronucleus.

The result that we have obtained that most closely achieves the objectives of the original proposal is the finding that long-term frozen sperm from E. coqui as well as several other species remains competent to initiate nuclear decondensation in cytoplasmic extracts prepared from E. coqui eggs. This result indicates that long-term frozen sperm from these frogs is largely undamaged, capable of initiating development, and that the initial steps at least are largely species independent. This result, in and of itself, gives significant support to the hypothesis that methods can be developed to resurrect extinct Eleutherodactylus species.

Major Accomplishments

• Artificial induction of ovulation for the production of large numbers of unfertilized eggs.

- Artificial induction of spermiation.
- Demonstration of nuclear decondensation from long-term frozen sperm.
- Sperm cryopreservation.
- In vitro fertilization and generation of viable frogs.
- Preliminary observations of induced egg activation.

Publications, Manuscripts, Abstracts

Michael, S.F., Buckley, C., Toro, E. and Vincent, S. Induced ovulation and egg deposition in the direct developing anuran *Eleutherodactylus coqui*. Submitted to Journal of Comparative Endocrinology.

Jones, C. and Michael, S.F. Cryopreservation of sperm from the terrestrial breeding anuran *Eleutherodactylus coqui*. Submitted to Cryobiology.

Michael, S.F., Toro, E., Gonzalez, J. and Garrett, T. Induced spermiation in the direct developing anuran *Eleutherodactylus coqui*. In preparation.

Toro, E., Reiser, J., Isern, S. and Michael, S.F. Production of transgenic frogs (*Eleutherodactylus coqui*) using a lentivirus vector. In preparation.

Presentations

Michael, S.F., West Indian Eleutherodactylus Frogs: Acoustic Communication and Conservation Issues. University of New Orleans. Department of Biology Seminar Series. 9/11/99

Intellectual Development

1. Student Names: Cecily Jones, Shawn Vincent, John Carlson, Sebastian Lourido, Jo Gonzalez, Esteban Toro

2. Period of funding:

Jones, Xavier SPRITE student 5/00-12/00

Vincent, Loyola undergraduate research assistant 5/00-4/01. Tulane EEOB graduate student 5/01-present.

Carlson, Tulane Tropical Medicine graduate student 8/99-present

Lourido, Tulane undergarduate research assistant 9/00-present

Gonzalez, Universidad Metropolitana, San Juan, PR. Research assistant 5/02-8/02

Toro, Universidad de los Andes, Bogota, Colombia. Research assistant 5/02-present

3. Brief description of duties and responsibilities:

Jones, Sperm cryopreservation studies.

Vincent Induced ovulation, in vitro fertilization and egg activation studies.

Carlson, In vitro fertilization, egg activation and nuclear decondensation studies

Lourido, In vitro fertilization and egg activation studies.

Useable Technologies: N/A

Effects of the Environment on Leiomyoma Cells and Women's Health

Principal Investigator:

Valerie Wilson, PhD. formerly

Clinical Associate Professor, Environmental Health Sciences

And Deputy Director

Center for Bioenvironmental Research at Tulane and Xavier Universities

Reporting Period:

May 2001 – April 2002

Primary Objectives of Research Activities

- Study genetic and biologic markers related to regulation of uterine function in women
- Study metabolic and hormonal differences
- Evaluate role of biologic and environmental factors

Progress Made to Achieve these Objectives

Ovarian steroid hormones play an important role in the growth of the uterine fibroids, or leiomyoma. The tumors grow during the reproductive years, increase in size during pregnancy, and recede after menopause. Studies suggest that leiomyoma cells are hypersensitive to estrogen. Therefore, treatment with gonadatrophin-releasing hormones or their analogs, which results in reduced hormone concentrations, leads to reduction in size of the leiomyoma. During this year, a variety of molecular studies were undertaken to explore the etiology of leiomyoma. Substantial results were made in studies related to leiomyoma and: 1) the role of methylation; 2) relationship of the Wnt-7a gene; 3) the expression of estrogen receptor (ER) β; and 4) the Hoxa gene.

Major Accomplishments

- This study identified and cloned two genomic fragments that frequently undergo methylation changes in leiomyoma. Nucleotide sequence analysis revealed no significant matches between the two fragments and known sequences in Genebank database. Further studies showed that the two BstU I sites in the fragment 2 were hypermethylated in 80% (12 of 15) of the leiomyomas. These two novel hypermethylated genes may be potential candidates for study of their role in leiomyoma pathogenesis.
- Wnt7a mRNA levels were lower in leiomyoma compared to paired myometrium in 55% of the samples in proliferative phase and in 56% of the samples in secretary phase. In general, Wnt7a mRNA levels showed a similar trend, that is, lower in leiomyoma compared to paired myometrium, in all three phases of the menstrual cycle. In conclusion, this research has demonstrated a decreased expression of Wnt7a in uterine leiomyoma as compared to that in the adjacent myometrium. The decreased expression has a reverse correlation with the ERα. The pathological appearance in leiomyoma resembles that of the myometrium with features of overgrown, poor differentiation. Wnt7a is the best candidate for studying the patterning control of the uterine myometria.

- We have compared the expression of estrogen receptor ER-α and ER-β mRNA between uterine leiomyoma and adjacent normal myometrium in 36 patients. In comparing the levels of expression of the two types of estrogen receptor, three conclusions were drawn:

 the expression of ER-α is higher than that of ER-β in both the leiomyoma and normal myometrium;
 the ER-α expression is increased in leiomyoma compared to that of the adjacent normal myometrium; and
 ER-β expression is the same or even lower in leiomyoma than in the adjacent normal myometrium. Based on our experiments,
 of the patients show an increased ratio of ER-α/ER-β in leiomyoma, and immunohistochemical staining of both ER-α and ER-β confirms the transcription-PCR (RT-PCR) assay results. We conclude that in addition to ER-α, ER-β may play an important role in the development of leiomyoma. Imbalance in expression of ER-α and ER-β may contribute to the pathogenesis of uterine leiomyoma.
- Diethylstilbestrol, or DES, is known as a chemical that mimics the actions of estrogen. Evidence suggests that mice, exposed to DES during early development, show abnormal uterine development. One target for change at the gene level is the Hoxa gene, and this gene is regulated by a specific initiation sequence known as the promoter. The principal effect of DES on DNA is to change the way that DNA is chemically modified. Our research indicates DES does not affect the DNA that codes for the promoter of the Hoxa gene. In other gene systems, DES does make modifications in the promoter, so therefore the mechanism of gene imprinting by DES is gene selective.

Publications

Li S, Ma L, Chiang, TC, Burrow, M, Newbold RR, Negishi M, Barrett JC, and McLachlan JA. Promoter CpG mehtylation of Hox-a10 and Hox-a11 in mouse uterus not altered upon neonatal diethylstilbestrol exposure. *Molecular Carcinogenesis* 32:213-219 (2001).

Li S, Chiang T-C, Davis GR, Williams RM, Wilson VP, and McLachlan JA. Decreased expression of Wnt7a mRNA is inversely associated with the expression of estrogen receptor α in human uterine leiomyoma. *The Journal of Clinical Endocrinology and Metabolism* 86 (1): 454-457 (2001).

Li S, Hursting SD, Davis BJ, McLachlan JA, and Barrett JC. Environmental exposure, DNA methylation, and gene regulation: lessons from diethylstilbesterol-induced cancers. *Annals of the New York Academy of Sciences* 983:161-169 (2003).

Li S, Chiang T-C, Barrett JC, and McLachlan JA. DNA hypomethylation and imbalanced expression of DNA methyltransferases (DNMT1, 3A, 3B) in human uterine leiomyoma, (in press)

Presentations

Dr. Shuanfang Li, "Deregulation of HOXA-10 expression in mouse uterus after neonatal exposure to Diethylstilbestrol (DES) is not associated with its promoter CPG methylation," 92nd Annual American Association for Cancer Research, New Orleans, Louisiana, March 24-28, 2001.

Intellectual Development

N/A

Useable Technologies

N/A

N00014-99-1-0763 A.24 Integrated Bioenvironmental Hazards Research Program

Acoustic Mapping of Sediment Storage in the Lower Mississippi and Atchafalaya Rivers

Principal Investigator:

Mead A. Allison, PhD

Department of Earth and Environmental Sciences

Tulane University

Reporting Period:

May 2001 – April 2002

Primary Objectives of Research Activities

To investigate the seasonal storage of sediment in the lower Mississippi River in Louisiana in order to determine the volumes, areas affected, and mechanisms of formation.

Progress Made to Achieve these Objectives

To date, we have made great strides in understanding the three issues mentioned above. We now know that sediment storage in the lower Mississippi takes the form of two distinct mechanisms. The first is found in the lowermost river (below mile 13 to about the mid-point of the three main passes at the river entrance into the Gulf of Mexico) and is associated with upriver penetration of the salt wedge from the Gulf in periods of low river discharge. During this period, estuarine circulation generates a turbidity maximum that leads to rapid deposition of fine-grained sediments. As long as the stratification is upriver of a particular point on the river bottom, any sediment stored on by this mechanism will remain until the salt wedge is moved downriver past the point with rising discharge. Radioisotopic profiles of sediment cores from the deposits show that deposition rates are mm/day and pore water salinity variations track variations in overlying water composition. Subbottom acoustic (CHIRP) maps have mapped out the extent of this storage and show that it is confined to the deepest part of the channel where saline waters are confined. The overlap between salt wedge extent and bottom sediment storage area has been confirmed by water column mapping with a CTD equipped with turbidity sensors. Maximum thickness exceeds 2 m in some locations during lowest discharge and total volume during a typical flood year is estimated at about 10% (10 million meric tons) of the annual Mississippi suspended load.

The second mechanism of river bottom sediment storage is a freshwater mechanism that has been mapped upriver to mile 181 but appears to not be active below the point of estuarine interference during a significant part of the flood cycle (mile 13). This storage is of less thickness (typically 10-50 cm), but has been recognized in sidescan sonar images from backscatter intensity differences, and from sediment cores. It also occurs during low discharge but is in the shallowest part of the channel. Bottom sampling and acoustic mapping show that it is confined to the 0-20 m depth interval. We believe that this is formed as a function of increasing bottom frictional bottom stress on the flow in shallow regions leading to an overall increase in flow velocity and the ability to transport sediments in suspension. As this water depth region encompasses 59% of the river bottom between miles 13 and 181, this storage mechanism is considerable. Estimates based on a 20 cm thick layer at low discharge are about 14 million metric tons) of the annual Mississippi suspended sediment load.

Major Accomplishments

Two research cruises were conducted on the R/V Eugenie in June 2001 and October-November 2001. Two 10 km reaches of the river were mapped at English Turn and Venice with multibeam bathymetry, sidescan sonar, and CHIRP subbottom sonar. A suite of surface sediment grab samples were also done for each grid on each field study. In addition, a number of 3-m-long gravity cores were collected from selected locations in the field areas.

- A third research cruise on the Eugenie was conducted in 3/02 specifically to collect long gravity cores at English Turn from areas where relict units were exposed in the river thalweg. These were used to estimate the character and volume of the contribution of relict material to the suspended load of the lower Mississippi.
- A variety of cores and grab sediment samples were analyzed for geochronology (²³⁴Th, ⁷Be, ²¹⁰Pb, ¹³⁷Cs), grain size, and pore water salinity.
- All multibeam and sidescan data was post-processed in CARIS 5.2 software and mapped in ArcView GIS software. Sediment storage maps were also created and placed in ArcView using CHIRP profiles.

Publications, Manuscripts, Abstracts

Draut, A.E., Kineke, G.C., Velasco, D.W., Allison, M.A., and Prime, R.J., submitted. Influence of the Atchafalaya River on recent evolution of the chenier plain inner continental shelf, northern Gulf of Mexico. *Continental Shelf Research*.

Galler, J.J., Bianchi, T.S., Allison, M.A., Campanella, R., and Wysocki, L., in press. Sources of aged terrestrial organic carbon to the Gulf of Mexico from relict strata in the Mississippi River. *EOS*, *Transactions of the American Geophysical Union*

Allison, M.A. and Neill, C.F., 2002. Accumulation rates and stratigraphic character of the modern Atchafalaya River prodelta, Louisiana. *Transactions of the Gulf Coast Association of Geological Societies*, 52:1031-1040. 4-7. Three other papers in preparation for submission in 2003.

Presentations

Allison, M.A. and Galler, J.J., 2003. River trends: transport and storage of mud and sand in the lower Mississippi River. Workshop on River Resource Management in the 21st Century, Louisiana Governor's Office of Coastal Activities. February.

Galler, J. and Allison, M.A., 2002. Estuarine and bathymetric controls on seasonal sediment storage in the lower Mississippi and Atchafalaya Rivers. Geological Society of America Annual Meeting, Denver, CO.

Neill, C.F., and Allison, M.A., 2002. Prodelta formation on the Atchafalaya Shelf, Louisiana. Geological Society of America Annual Meeting, Denver, CO.

Allison, M.A. and Neill, C.F., 2002. Accumulation rates and stratigraphic character of the modern Atchafalaya River prodelta, Louisiana. *Transactions of the Gulf Coast Association of Geological Societies*, Austin, Texas.

Allison, M.A. and Coakley, B., 2001. Seasonal and interannual storage of fine-grained sediment in the lower Mississippi River: evidence from acoustic mapping and bottom sampling. *ASLO Alberquerque*.

Campanella, R., Coakley, B., and Allison, M.A., 2001. Historical bathymetry from the Army Corps of Engineers for the lower Mississippi River, 1915-1992. Evolution of a controlled river. *ASLO Alberquerque*

Coakley, B. and Allison, M.A., 2001. Repeat swath mapping at English Turn: direct imaging of bedform migration in the lower Mississippi River. ASLO Alberquerque.

Intellectual Development

- 1. Student(s) Name: John J. Galler, Ciara R. Neill
- 2. Funding Period: April 2001-April 2002
- 3. **Duties and Responsibilities**: John Galler was a full participant in the project including field data collection and laboratory analysis. John is utilizing this data as the means to produce his PhD dissertation (anticipated in 2004). Data collected on the Atchfalaya shelf in transit between the riverine field areas is being utilized by Neill as part of her M.S. thesis research. A number of other graduate and undergraduate students participated in the field phase and were exposed to the technologies used in the study.

Useable Technologies

N/A

Roles of Natural Organic Matter (NOM) on the transport of colloidal particles

Principal Investigator:

Elia Eschenazi, PhD.

Associate Dean, Physics and Engineering

Xavier University of Louisiana

Co-Investigator:

Kyriakos Papadopoulos, PhD. Associate Dean, Engineering

Tulane University

Reporting Period:

May 2001- April 2002

Primary Objectives of Research Project

The objectives of this project were:

To have a better understanding the stability of subsurface colloidal particles which is vital in predicting their transport. The response of a subsurface aquifer to either natural or man-made disturbances can cause changes in such variables as pH or ionic strength, each of which can significantly impact the interaction forces between particles. Furthermore, the behavior of an NOM coating in a given environment will depend on its specific size and composition. Understanding the stability of colloidal particles in such diverse conditions is a critical path in developing a generalized model for predicting the fate and transport of subsurface contaminants.

In previous research, we studied the effects of divalent ions and humic acid on the transport of colloids through porous media using packed bed. Atomic force microscope (AFM) studies were conducted on the adsorption of HA and/or colloidal particles to mica surface. This project will continue our investigation on the role of NOM on the transport of colloidal particles. Microcapillary video microscope, will be utilized to visualize the flow of colloids in porous media. In-situ study on the adsorption of HA and/or colloids is a better simulation to subsurface flow situation. The effects of the steric interaction between colloids coated with NOM will be studied by measuring the force by the AFM.

Progress Made to Achieve these Objectives

Our research¹⁻⁴ in this and other projects demonstrated that humic acids facilitated colloid-facilitated transport of contaminants in soil, whereas Calcium ion has a profoundly hindering effect against such transport, thus countering the action of humic acids.

Based on the theory of colloidal interactions, we believe that trivalent ions, such as Al3+, will have an even grater effect than the divalent Ca2+. We planed therefore to conduct AFM studies on the attachment of colloids on mica substrates that mimic the surface of soil. These studies aim to reveal how Al3+ works in the presence of varying concentrations of humic acids typical of contaminated soils.

The attachment of iron oxide colloidal particles on a mica surface in the presence of humic acid (HA) and/or aluminum ions (Al3+) was investigated with atomic force microscopy (AFM). In the absence of both HA and Al3+, adhesion of some individual colloidal particles onto the solid substrate increased with

time as clusters formed and grew. HA substances present in the colloidal suspension considerably reduced the attachment of iron oxide colloidal particles onto the mica surface. In addition, most attached colloidal particles tended to aggregate following long immersion times forming big clusters on specific areas of the mica sheet, thus displaying a selective way of attaching to the mica surface. The presence of Al3+ greatly increased the attachment of iron oxide colloidal particles whether in the absence or in the presence of HA. These observations carry significance in the colloid-facilitated transport and mobilization of contaminants in subsurface environments, where humic substances have previously been known to enhance transport while polyvalent metal ions are noted as promoters of particle aggregation in suspensions and slurries. A paper on these studies is in preparation and it will be submitted for publication (5).

In future work other trivalent ions, like Fe3+, will be investigated as well in order to determine weather it is purely the valence that causes particle attachment or weather such attachment may also be ion specific.

An immediate use of our results will be in the choice of sites for waste disposal. Another use may be found in the design of soil remediation techniques.

Publication, Manuscripts and Abstracts

Lixiong Wen, Rong Chang Wu, E.Eschenazi, and K. Papadopoulos, AFM of Amidine Latex Particle Attachment on Mica, Colloids and Surfaces A (2001), in press.

Christina J. Davis, E.Eschenazi, , K. Papadopoulos, Effect of Divalent Ions and Humic Acids on the Transport of Colloids through Porous Media, Colloids and Polymer Science, (2001), in press.

A.Liu, R.C. Wu, E. Eschenazi, K. Papadopoulos, *AFM on humic acid adsorption on mica*, Colloids and Surfaces A, 174,245-252, (2000).

S.R. Dedhiikan, Elia Eschenazi, Kyriakos Papadopoulos Transport of Colloids Through Porous Beds in the Presence of Natural Organic Matter, Colloids and Surfaces, Vol 145, 93 -100,(1998).

M. Gibbs, E. Eschenazi and K. Papadopoulos, AFM of iron oxide particles attachment on mica, in preparation.

Presentations

None

Intellectual Development

N/A

Useable Environmental Technologies

N/A

Sr and Pb Isotope Studies of Mississippi Delta Estuarine Waters & Sediments

Principal Investigator:

Franco Marcantonio, PhD.

Associate Professor

Department of Earth and Environmental Sciences

Tulane University

Reporting Period:

May 2001 - April 2002

Primary Objectives of Research Project

Project 1) Further definition of the mechanisms of removal and/or labilization of strontium and other trace elements (i.e., Mo, V, U, Cu, Pb, Zn, Fe, Mn) in the estuarine mixing zone of the Mississippi River.

Project 2) We intend to answer the following questions: Do sediments deposited by the Mississippi River effectively integrate the Pb pollution signature of the US? Will this isotopic signature be unique and, hence, provide us with an important chronological tool?

Progress Made to Achieve these Objectives

Project 1) Dissolved (<0.2μm) and particulate (>0.2μm) Mn, Fe, U, V, Mo and Ba were studied in the Mississippi River mixing zone. Samples were collected during four cruises from 1999-2002. Each cruise was representative of different seasons and/or flow stages. Some water depth profiles were also examined.

Project 2) Four sediment vibracores were collected from the Terrebonne and Mississippi Delta wetlands. All four have been analyzed for Pb isotopes.

Major Accomplishments

Project 1)

- the variability patterns of the surface dissolved concentrations along salinity gradients for each season are very similar for U, Mo and Ba, and roughly similar for Fe, Mn and V
- dissolved U, Mo, and V concentrations are relatively high in the sampled riverine endmember during low discharge and low during high discharge periods
- maximum dissolved Mn and Fe concentrations are found in the spring high discharge period
- trace metal contents of the particulates in surface water, except Mo, decrease, from river to the sea in general
- the degree of mixing, particle dynamics, effect of benthic flux, biological-associated cycling, upwelling and advection of shelf waters, and drainage area characteristics are potential causes for the spatiotemporal variations

Project 2)

• Pb isotope ratios in four cores from throughout the lower river wetlands show similar variations with time

- we interpret the similar variability as indicative of similar sources of Pb pollution emissions being deposited at similar times
- the potential for using such variations as a chronometer needs to be verified

Publications

Xu, Y and Marcantonio, F. (2003) Spatiotemporal variations in trace metals in the Mississippi River mixing zone: insights into estuarine processes, submitted to *Chemical Geology*.

Presentations

Xu, Y. and Marcantonio, F. (2001) Sr and trace metals in the Mississippi River mixing zone, *Geophys. Union Trans.*, 82, F648.

Intellectual Development

- 1. Student Name: Yingfeng Xu (PhD student)
- 2. **Period of funding**: Xu funded by this grant from 4/01 4/02
- 3. Duties and Responsibilities: Xu responsible for geochemical measurements in Project 1.

Useable Environmental Technologies

N/A

The Effect of Particle Dynamics on Rough Surfaces Formed by Sedimenting Particles

Principal Investigator: Kathleen McCloud, PhD.

Associate professor and Chair

Department of Physics and Dual Degree Engineering

Xavier University of Louisiana

Co-Investigator: Elia Eschenazi, Professor, PhD.

Department of Physics and Dual Degree Engineering

Xavier University of Louisiana

Reporting Period: May 2001 - April 2002

Primary Objectives of Research Project

In the experimental work, the interaction between the hydrodynamics in the sedimenting particles and the resulting roughness and growth of the surface will be studied, using the scaling ansatz proposed by Family and Vicsek [1]. In this ansatz, the rms thickness of the interface is defined to be:

$$W(L,t) = [1/N \Sigma h(x_i,t)^2]^{1/2}$$

where $h(x_i,t)$ is the difference between the average height and the actual height at some position x_i . The scaling ansatz predicts that:

$$W(L,t)=L^{\alpha}f(t/L^{\alpha/\beta})$$

where the exponents α and β are the static and dynamic scaling exponents. The function $f(t/L^{\alpha/\beta})$ is expected to have an asymptotic form such that

$$\label{eq:weights} \begin{split} W(L,t) \sim t^{\beta} & \text{ for } t {<\!\!<} L^{\alpha/\beta} \\ W(L,t) \sim L^{\alpha} & \text{ for } t {>\!\!>} L^{\alpha/\beta} \end{split}$$

The roughness and dynamic exponents have been argued to be universal, and are expected to depend on the dimension of the system under study and the interactions between the sedimenting particles.

The average velocity, velocity fluctuations, and path of sedimenting particles can be examined experimentally using a standard video camera. In the proposed work, sedimentation will take place at very low Reynolds' numbers, in viscous fluids such as heavy paraffin oil. In this way, the dynamics between particles can take place at long enough times to be visible using the video camera. The path of individual particles will be tracked as they fall, and afterwards. In the proposed project, the dimensions of the system under study is given by the ratio of particle size to gap size, which can be changed easily by changing the thickness of the Teflon spacers in the open cell. The 0.6 mm particles will then be deposited at a single, controlled deposition rate, and photographs will be taken of the interface as it builds. The pictures will be scanned, and the interface traced using the freeware software program NIH Image. The rms thickness of the interface will be found, and the static and dynamic roughness exponents and the cross-over length scale will be extracted from the scaling relations [1]. Different gap sizes will be N00014-99-1-0763

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studied, spanning a range from 0.8 mm, where gap is just large enough to allow the particles (diameter 0.6 mm) to sediment without getting stuck between the plates, to 1.4 mm where the gap size is more than twice the diameter of the beads and the system is almost three dimensional. There is a limit to the extent over which the gap can be widened without changing the method of analysis, since at one point it will no longer be possible to analyze the rough surface as a one-dimensional interface. However, this range of gap values should give a reasonable idea of the effect of bringing the system closer to a three-dimensional system.

Progress Made to Achieve these Objectives

The experiments take place in sedimentation cells consisting of glass plates separated by a small gap set by Teflon spacers. Glass beads of 0.6 mm diameter are allowed to sediment between the plates to form a rough surface at the bottom. The beads are deposited into the cell from above, and the gap in the cell can be set to study different ratios of gap/ particle size (R). We have studied the effect of changing the gap between the plates to a limit of about twice the diameter of the beads. If we continue to accept the scaling ansatz proposed by Family and Viscek [1], we find that the roughness exponent is robust to the changes in the separation between the walls of the container [2]. There is a slight increase in the roughness exponent at R = 2, which is under further investigation using computational methods to simulate the effects of the wall separation, as well as direct experimental investigations of correlations between particles in the fluid. However, the data also show evidence of continuous change with L, so that the possibility that scaling arguments do not hold must be taken seriously.

We also started focusing on a new issue – the stability of the surface during deposition. The assumption has been that, once deposited, the beads roll to a local minimum and are stable at that position. However, upon closer examination of the deposited beads during the deposition process, it has become clear that under some circumstances there can be fairly substantial movement of either individual beads or clusters of beads. The beads will be at rest for long time periods (compared to the time for the entire surface to be built), and the movement will be somewhat sudden. These beads can be deep in the already formed surface at the time of the movement. We are currently investigating the effect this movement will have on the roughness of the surface, since to this point we have considered the interactions between the particles as they sediment to be the main source of the roughness. It is not expected that this should be a large effect, as not many particles are involved and the movements are relatively rare (one or two per experiment) but we are working to quantify the effect on the final interface.

- [1] Family, F. and T. Vicsek, Journal of Physics A 18, L75 (1985)
- [2] McCloud, K, and M. Kurnaz, International Journal of Modern Physics B 16, 1217 (2002)

Major Accomplishments

- Measurements of the roughness exponents have been made for systems of gap/particle diameter ratios
 up to slightly greater than 2, with no significant change in the roughness exponent.
- In these same systems, there was no significant change in the crossover length scale between the two observed roughness exponents.
- The data show some signs of continuous change with the length scale L, which is at least an indication that scaling may not be valid and that alternate methods of analysis should be explored.

• There is evidence that the interfaces undergo some local shifts while sedimentation is taking place, originating from shifts in beads located deep in the surface. These shifts are fairly rare but must be examined more thoroughly for their effect on the final shape of the interface.

Publications, Manuscripts, Abstracts

McCloud, K, and M. Kurnaz, International Journal of Modern Physics B 16, 1217 (2002)

Presentations

McCloud, K. The Effect of Hydrodynamics on the Roughness of Surfaces Formed by Sedimenting Particles", poster presentation by Herbert Brown at the National Conference of Black Physicists in Huntsville, Alabama, March 15, 2002.

McCloud, K. "Interactions between Sedimenting Particles", presentation by Gregory Bogin at the National Conference of Black Physicists in Huntsville, Alabama, March 15, 2002.

Intellectual Development

- 1. Student Name: Herbert Brown
- 2. **Period of Funding:** 4/01-4/02
- 3. **Duties:** Responsible for data acquisition and measurement for particles interacting during sedimentation
- 1. Student Name: Gregory Bogin
- 2. **Period of Funding**: 4/01-4/02
- 3. **Duties:** Responsible for data acquisition and measurement for the behavior of particles at the interface before and during sedimentation. Also responsible for helping mentor and train other students in the lab.
- 1. Student Name: Merideth Robinson
- 2. **Period of Funding**: 6/01-4/02
- 3. **Duties:** Responsible for measurements of particle volume fraction and density during sedimentation, and the resulting effects on the interface.
- 1. Student Name: Paula Myers
- 2. **Period of Funding:** 6/01-4/02
- 3. **Duties:** Responsible for video analysis and setup of sedimentation cells for a variety of sedimentation experiments.

Useable Environmental Technologies

N/A

River-Ocean Interactions (Phase I): The Processing and Fates of Nutrients and Organic Carbon from the Mississippi River

Principal Investigator:

Brent McKee, PhD.

Professor

Department of Earth and Environmental Sciences

Tulane University

Co-Investigator(s):

Tom Bianchi, PhD.

Associate Professor

Department of Earth and Environmental Sciences

Mike Dagg, PhD.

Professor

Louisiana Universities Marine Consortium

Richard Miller, PhD.

Chief Scientist

NASA Stennis Space Center

Rodney Powell, PhD. Assistant Professor

Louisiana Universities Marine Consortium

Reporting Period:

May 2001 – April 2002

Primary Objectives of Research Project

To use a combination of remote sensing, biological, and geochemical techniques to determine the major pathways for terrestrial carbon from the Mississippi River and marine carbon produced from nutrients delivered by the Mississippi River.

Progress Made to Achieve these Objectives

Great progress was achieved for this "proof-of-concept" project. The major pathways for terrestrial carbon from the Mississippi River have been determined for two seasonal time frames in 2000 (Springhigh flow; Fall low flow). These two field experiments have resulted in a large volume of data that was collected by all five investigators under strict conditions of coordination so that a major portion of the carbon pathway can be understood. A number of high quality manuscripts were produced and published in peer-reviewed journals.

Major Accomplishments

- Web page (<u>www.lumcon.edu/mirir</u>) was maintained to report our major finding to the public, share our data with other researchers and to enhance data sharing between PIs.
- The Second Data workshop held at Tulane in May 2001 to begin the process of achieving a coordinated understanding of carbon pathways in the study area. Results from the Second major

- field effort (successfully completed in October 2000; a 10-day research cruise to characterize the Fall, low discharge, season in the study area) were presented and discussed.
- McKee and Bianchi co-chaired an all-day session at a major national meeting (ASLO; American Society of Limnology and Oceanography) focused on river-ocean processes. McKee presents a talk that outlines the initial overall synthesis of the project. Five other posters were presented detailing findings of the project.

Publications, Manuscripts, Abstracts

- Chen, N., T. Bianchi, B. McKee, and J. Bland. 2001. Historical Trends of Hypoxia on the Louisiana Shelf: the Application of Pigments as Biomarkers. *Organic Geochemistry*. 32 (4): 543-561 2001
- Bianchi, T, S. Mitra, and B. McKee. 2001. Sources of Terrestrially derived Organic Carbon in Lower Mississippi River and Louisiana Shelf Sediments: Implications for Differential Sedimentation and Transport at the Coastal Margin, *Marine Chemistry* 77 (2/3): 211-223.
- Mitra, S., T. Bianchi, B. McKee, and M. Sutula. 2002. Black Carbon from the Mississippi River: Quantities, Sources and Potential Implications for the Global Carbon Cycle. *Environmental Science and Technology* 36: 2296-2302.
- McKee, B.A., R.C. Aller, M.A. Allison, T.S. Bianchi, and G.C. Kineke. 2003. Transport and Transformation of Dissolved and Particulate Materials on Continental Margins Influenced by Major Rivers: Benthic Boundary Layer and Seabed Processes. *Continental Shelf Research*. (In Press)
- Dagg, M.J. and S.L. Brown. 2003. The potential contribution of fecal pellets from the larvacean *Oikopleura dioica* to vertical flux of carbon in a river dominated coastal margin. G. Gorsky (ed), *Response of marine Ecosystems to Global Change: Ecological Impact of Appendicularians*. Gordon and Breach (In Press).
- Corbett, R., B. McKee, and D. Duncan 2003. An evaluation of mobile mud dynamics in the Mississippi River Deltaic region. *Marine Geology*. (Accepted)
- Liu, H., M. Dagg, L. Campbell and J. Urban-Rich. 2003. Picophytoplankton and bacterioplankton in the Mississippi River plume and its adjacent waters. *Estuaries* (Accepted)
- Liu, H. and M. J. Dagg. 2003. Interactions between nutrients, phytoplankton growth, and grazing by micro- and meso-zooplankton in the plume of the Mississippi River. Mar. Ecol. Prog. Ser. (Accepted)
- Chen' N., T. Bianchi and B. McKee (Submitted) Early Diagenesis of Chlorophyll-a in the Lower Mississippi River and Louisiana Shelf: Implications for Carbon Cycling in a River-Dominated Margin. *Geochimica et Cosmochimica Acta*. (Submitted)

Sutula, M., T.S. Bianchi, and B.A. McKee. (Submitted) Lower Mississippi River seasonal sediment storage, diagenesis, and removal: implications for phosphorous cycling in the Mississippi River and Gulf of Mexico. (Submitted) *Limnology and Oceanography*.

Miller, R. (Submitted) Measuring CDOM using a Multiple Pathlength Liquid Waveguide System. *Continental Shelf Research.* (Submitted)

In Preparation:

Chen, N., T. Bianchi, and J. Bland. Novel sediment pigment biomarkers as indicators of grazing on the Louisiana shelf: The application of high-performance liquid chromatography-mass spectrometry (HPLC-MS) techniques. *Marine Chemistry*. In Preparation.

Corbett, R., B. McKee and D. Duncan. The fate of particle-reactive radionuclides on a river-dominated margin: The Mississippi River and adjacent shelf. *Geochimica et Cosmochimica Acta*. In Preparation

Wysocki, L., T. Bianchi, and B. McKee. Sources and spatial variability of terrestrial organic matter in sediments within the depositional flow path of the Mississippi River Plume. *Geochimica et Cosmochimca Acta* In Preparation

Wysocki, L., T. Bianchi, R. Miller, and R. Powell. Spatial dynamics of particulate and dissolved organic carbon in the Mississippi River Plume: Effects of flow regime. *Limnology and Oceanography*. In Preparation

Wysocki, L., and T. Bianchi. Spatial variability in the composition and relative abundance of amino acids in the total nitrogen pool within the Mississippi River Plume. *Limnology and Oceanography*. In Preparation

Powell. R. Distribution of Fe complexing ligands in the Mississippi River Plume. Estuarine and Coastal Shelf Science In Preparation

Powell, R. and A. Finelli-Wilson. Nutrient distributions in the Mississippi River Plume. Estuaries In Preparation

Miller, R. C. Hall, C. Del Castillo, and B. McKee. Bio-optical properties associated with the Mississippi River plume. *Journal of Geophysical Research* In Preparation

Hall, C., R. Miller and B. McKee. Relationship between the Fluorescence Lifetime of Chlorophyll a and Primary Productivity within the Mississippi River Plume. *Journal of Plankton Research*. In Preparation

Miller, R., C. Hall and S. Fernandez. Estimates of Phytoplankton Photochemical Efficiency Derived from Fluorescence Lifetime Measurements *Journal of Plankton Research* In Preparation

Urban-Rich, J. and M.J. Dagg. The potential contribution of mesozooplankton fecal pellet production to carbon flux in the vicinity of the Mississippi River plume during spring and fall 2000. *Marine Ecology Progress Series*. In Preparation

Presentations

- B. McKee, M. Dagg, T. Bianchi, R. Corbett, R. Powell. Carbon Cycling and Burial in the Mississippi Delta Region. 2001 Aquatic Sciences Meeting sponsored by the American Society of Limnology and Oceanography; Albuquerque NM.
- N. Chen, T. Bianchi and B. McKee. Fate of Chlorophyll-A in the Lower Mississippi River and Louisiana Shelf: Effects of redox. 2001 Aquatic Sciences Meeting sponsored by the American Society of Limnology and Oceanography; Albuquerque NM.
- M. Satula, T. Bianchi and B. McKee. Effect of Seasonal Sediment Storage and Diagenesis in the Lower Mississippi River bed on Bioavailability of Particulate Phosphorus Flux to the Gulf of Mexico. 2001 Aquatic Sciences Meeting sponsored by the American Society of Limnology and Oceanography; Albuquerque NM.
- R. Miller, C. Hall, C. Del Castillo, J. Yuan, B. McKee and M. Dagg. Bio-optical Properties of the Mississippi River Plume and Adjacent Shelf, 2001 Aquatic Sciences Meeting sponsored by the American Society of Limnology and Oceanography; Albuquerque NM.
- A. Wilson-Finelli and R. Powell. Nutrient Cycling in the Mississippi River Plume. 2001 Aquatic Sciences Meeting sponsored by the American Society of Limnology and Oceanography; Albuquerque NM.
- M. Dagg and H. Liu. Microzooplankton Grazing of the Phytoplankton in the Mississippi Plume. 2001 Aquatic Sciences Meeting sponsored by the American Society of Limnology and Oceanography; Albuquerque NM.
- R. Powell and A. Wilson-Finelli. Organic Complexation and Speciation of Iron in the Mississippi River Plume. 2001 Aquatic Sciences Meeting sponsored by the American Society of Limnology and Oceanography; Albuquerque NM.
- R. Corbett. B. McKee and D. Duncan. Naturally-Occuring Radioisotopes as Indicators of Sediment Cycling in River-Dominated Ocean Margins. 2001 Aquatic Sciences Meeting sponsored by the American Society of Limnology and Oceanography; Albuquerque NM.

Intellectual Development

- 1. Student Names: Laura Wysocki, Mike Stewart
- 2. Period of funding: April 2001 March 2002
- 3. **Brief description of duties and responsibilities**: collect samples during both field experiments, perform analyses on organic constituents

Useable Environmental Technologies N/A

Autonomous Monitoring and Visualization Technology Development for Aquatic Environments

Principal Investigator:

Douglas J. Meffert, PhD.

Clinical Associate Professor and

Deputy Director

Center for Bioenvironmental Research

Co-Investigators:

George Rey

President

COTS Technology, LLC

Funding Period:

May 2001 - April 2002

Primary Objectives of Research Project

The increased prevalence and threat of chemical warfare agents have necessitated advanced warning of the presence of these harmful constituents in the air, water, and soil. The Tulane/Xavier Center for Bioenvironmental Research has been developing for the past several years biosensors to detect the compounds of interest to the Navy. The CBR has employed a variety of innovative biologically based receptors, which utilize biologic reactions to assess and report the types and quantity of toxins in the field. These projects employ a variety of methods including field studies and in vitro and in vivo laboratory studies at Tulane and Xavier Universities with some of the projects already employing initial field demonstrations.

The significance of this research includes development of new technologies, which will allow for cost-effective assessments of toxicity in aquatic environments of interest to the DOD. A remaining need for this program is the integration and co-development of technologies to deploy these biosensors in remote environments. The safest and most cost-effective means in aquatic environments would be through autonomous/unmanned underwater vehicles (AUV's). Our goal is to become the first entity in the nation to produce a biosensor for effective deployment on an AUV (tentatively scheduled for Fall 2004).

Progress Made to Achieve These Objectives

We have made significant progress in realizing our goal of having the first biosensor deployed on an AUV. The CBR has continued its partnership with NAVOCEANO to provide linkages and assistance in research development (i.e., modeling and field testing). Through this relationship, we have continued collaborations with Woods Hole Oceanographic Institute to ensure that our biosensor will be compatible with their REMUS AUV, so this can be used as a platform for one of our antibody-based biosensors (being developed by Drs. Diane Blake and Robert Blake of Tulane and Xavier, respectively).

For this project, I sought support to hire a computer graphics technician in the CBR's visualization laboratory to provide graphic support for computer/Internet visualization of aquatic monitoring results made possible through AUV modeling and field testing through this project. To date, we have established a website (www.leag.org) where we will ultimately be displaying this visualization.

Major Accomplishments

Facilitated interactions to promote coordinated research between Tulane and Xavier investigators; Naval Oceanographic Office, Stennis, MS; and private sector (COTS Technology, LLC and Sapidyne Instruments) for joint research on biosensor coupling with autonomous underwater vehicle navigation enhancement and "intelligent" programming for long-term deployment, accuracy, and self-regulation of biosensors in aquatic environments.

Publications, Manuscripts, Abstracts

Meffert, D.J., D.A. Blake, R.C. Blake III, R.G. Rey: Antibody, Cell-Based, and Transgenic Fish Biosensors for Autonomous Monitoring of Environmental Toxicants. Proceedings from the 12th International Symposium on Unmanned Untethered Submersible Technology. Sponsored by the Autonomous Undersea Systems Institute (AUSI), Durham, NH, August 27-29, 2001.

Presentations

Meffert, D.J., D.A. Blake, R.C. Blake III, R.G. Rey: Antibody, Cell-Based, and Transgenic Fish Biosensors for Autonomous Monitoring of Environmental Toxicants. Invited presentation at the 12th International Symposium on Unmanned Untethered Submersible Technology. Sponsored by the Autonomous Undersea Systems Institute (AUSI), Durham, NH, August 27-29, 2001.

Meffert, D.J., J.A. McLachlan, J. Fox, M. Schleuter, D. Blake, R. Blake III, and C. Gulledge: Integrated Biomarker and Biosensor Technologies for Long-term Stewardship: Promise and Uncertainties. Oral presentation at the Technical Information Exchange (TIE) Workshop in Albuquerque, NM, November 12-14, 2001.

Intellectual Development

N/A

Useable Environmental Technologies

- 1. **Title of technology product**: Integrated autonomous immunosensor and autonomous underwater vehicle system.
- 2. Description of technology product: See Accomplishments described above.
- 3. Utility/benefit/ROI/payoff of technology product: Enhanced real time biosensor deployment for environmental compliance and, ultimately biologic warfare detection.
- 4. **Timeline (demonstration, validation, completion, etc.):** AUV/biosensor integration to be completed in August 2004. Biosensor to be deployed on AUV or stationary buoys and AUV's subsequent to August 2004, pending acquisition of REMUS AUV.
- 5. Partners (academia, industry, labs/centers, federal agency, etc.): Naval Oceanographic Office, Tulane and Xavier Universities, Sapidyne Instruments, Boise, Idaho, COTS Technology, LLC, New Orleans, LA, Woods Hole Oceanographic Institute, Woods Hole, Massachusetts
- 6. Patents (applied for and issued): Patents will be applied for by partners.

Eugenie Field Core

Principal Investigator:

Douglas J. Meffert, PhD Clinical Associate Professor

Deputy Director

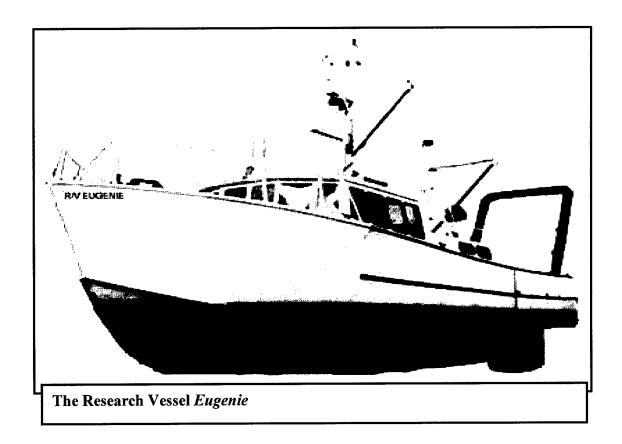
Center for Bioenvironmental Research

Funding Period:

May 2001 - April 2002

Primary Objectives of Research Project

The *RV Eugenie* was obtained by the CBR in April 2000 through a grant from the Coypu Foundation. The vessel provides a unique floating laboratory and observatory to support CBR continuing research efforts to address the scientific and social issues associated with the Mississippi River and Gulf of Mexico. The *RV Eugenie* is used primarily for measuring river concentrations of natural and man-made chemicals in the Mississippi River and its related ecosystems. To date, the research projects utilizing the *RV Eugenie* have been funded by the Office of Naval Research. ONR-funded investigators include Drs. Brent McKee, Tom Bianchi, Franco Marcantonio, and Mead Allision from Tulane's Departments of Geology and Ecological and Evolutionary Biology. We anticipate that future work will also be funded by the U.S. Departments of Interior and Commerce as well as investments from a diverse group of researchers throughout the country eager to study Gulf South ecosystems.



SPECIFICATIONS

*Length Overall: 60 feet *Draft: 8 feet, 1 inches *Beam: 16 feet, 7 inches *Tonnage: 50 gross tons *Hull Material: Steel

*Cruising Speed: 9 knots

*Minimum Speed: 1.8 knots
*Accommodations: 6 berths, galley,

head, shower, air conditioned sleeping compartment

*Potable water capacity: 300 gallons

*Propulsion: Detroit Diesel

12V-71N 400 horsepower, Twin Disc 3:1 transmission, turns 36" 4 bladed propeller

*Electrical: Northern Lights 20KW diesel generator 110V/220V AC single phase, Onan 8KW diesel generator 110V/220V AC single

phase, 32V DC 65 amp alternator, 12V DC 80 amp alternator

MAIN DECK

*Main Winch: Hathaway inline double drum trawl winch, line pull 2,000 pounds @ 40 rpm, with 1000 feet of 5/16-inch

7x19 stainless steel wire

*Gantry: 2000lb capacity U-frame hydraulic gantry, 3 block positions, min height clearance to deck 10'

*Cargo Boom: 1000 lb. capacity, 20'

clearance off deck, 16' long; *Hydrographic Davit: light duty electric winch, 300' 3/16" 7x19 stainless steel wire

*Work deck pilot station

*340 sq. foot work area, 16' clear deck

Progress Made to Achieve These Objectives

Summarized below are the research cruises on the R/V Eugenie during the project period covered in this productivity report.

Falling Discharge stage (late July to early September 2001): total 3 days

• Scheduled: 1 day (31 July) McKee et al.

Depart from COE docks to test Contrifuge underway

• Planned: 2 day cruise w/McKee et al

N.O. to Venice to samples Permanent Stations 1 and 2 (PS1 & PS2)

Low Discharge stage (October through November 2001): total 26 days

• Leg 1

Bianchi et al.: Duration: 4 days

Location: Round trip N.O. to B.R.

Activities: Water column sampling (surface and bottom)

EQ needed: CTD (possibly grab sampler and box corer -see note)

Notes:

Possible that trip up to (or back from) B.R. will involve Allison et al. Activities: Seismic mapping and grab sampling (this should be possible within the 4-day Bianchi et al. leg)

• Leg 2

Allison et al.: Duration: 10 days

Location: Downriver from English Turn to Venice Activities: Seismic mapping and grab sampling

EQ needed: CTD and grab sampler

McKee et al.: Duration: 2 days

Location: Downriver to Venice

Activities: Collect cores at PS 2 (Venice); Contrifuge sampling

EQ needed: CTD, grab sampler and box corer

Leg 3

Marcantonio et al.: Duration: 2 days

Location: Downriver from Venice to open shelf

Activities: Water column sampling along salinity gradient (surface with bottom samples for each station; 4 stations with depth profiles); 2 boxcores on the shelf for porewaters

EQ needed: CTD, grab sampler and box corer

• Leg 4

Allison et al.: Duration: 8 days

Location: Atchafalaya system

Activities: Seismic mapping and grab sampling

EQ needed: CTD and grab sampler

Note: possible box coring (McKee et al.)

Rising Discharge stage (December 2001 through February 2002): total 8 days

• Leg 1:

Bianchi et al.: Duration: 4 days

Location: Round trip N.O. to B.R.

Activities: Water column sampling (surface and bottom)

EQ needed: CTD (possibly grab sampler and box corer -see note)

Notes:

Possible that trip up to (or back from) B.R. will involve Allison et al.

Activities: Seismic mapping and grab sampling (this should be possible within the 4-day Bianchi et al. leg)

• Leg 2

McKee et al.: Duration: 2 days

Location: Downriver to Venice

Activities: Collect cores at PS 2 (Venice); Contrifuge sampling

EQ needed: CTD, grab sampler and box corer

Leg 3

Marcantonio et al. Duration: 2 days

Location: Downriver from Venice to open shelf

Activities: Water column sampling along salinity gradient (surface with bottom samples for each station; 4 stations with depth profiles); 2 boxcores on the shelf for porewaters

EQ needed: CTD, grab sampler and box corer

High Discharge stage (March through early May 2002): total 26 days

• Leg 1:

Bianchi et al.: Duration: 4 days

Location: Round trip N.O. to B.R.

Activities: Water column sampling (surface and bottom)

EQ needed: CTD (possibly grab sampler and box corer –see note)

Notes:

Possible that trip up to (or back from) B.R. will involve Allison et al.

Activities: Seismic mapping and grab sampling (this should be possible within the

4-day Bianchi et al. leg)

• Leg 2

Allison et al.: Duration: 10 days

Location: Downriver from English Turn to Venice Activities: Seismic mapping and grab sampling

EQ needed: CTD and grab sampler

McKee et al.: Duration: 2 days

Location: Downriver to Venice

Activities: Collect cores at PS 2 (Venice); Contrifuge sampling

EQ needed: CTD, grab sampler and box corer

• Leg 3 (tentative)

Marcantonio et al. or McKee et al.

Duration: 2 days

Location: Downriver from Venice to open shelf

Activities: Water column sampling along salinity gradient (surface with bottom samples for each station; 4 stations with depth profiles); 2 boxcores on the shelf for porewaters

EQ needed: CTD, grab sampler and box corer

• Leg 4

Allison et al.: Duration: 8 days

Location: Atchafalaya system

Activities: Seismic mapping and grab sampling

EQ needed: CTD and grab sampler

Note: possible box coring (McKee et al.)

One Day cruises to collect river water in the COE dock area: ~ 9 days (~ 1 day per month)

Major Accomplishments

- For major accomplishments, refer to productivity reports of Dr. McKee, Bianchi, Allison, and Marcantonio included elsewhere in this report.
- The CBR partnered with COTS Technology, LLC for the operation of the R/V Eugenie

Publications, Manuscripts, Abstracts

None

Presentations

None

Intellectual Development

N/A

Useable Environmental Technologies

N/A

Occurrence and Fate of Pharmaceutically Active Compounds and Other Emerging Contaminants

Principal Investigator:

Douglas J. Meffert, PhD.

Clinical Associate Professor and

Deputy Director

Center for Bioenvironmental Research

Co-Investigators:

Glen R Boyd, Ph.D., P.E.

Assistant Professor

Department of Civil and Environmental Engineering

School of Engineering Tulane University

Deborah Grimm, Ph.D.

Director, Coordinated Instrumentation Facility

Tulane University

Reporting Period:

May 2001 - April 2002

Primary Objectives of Research Project

A certain group of chemicals has been receiving increased attention recently. It is the pharmaceuticals and personal care products (PPCP) and endocrine disrupting chemicals (EDC). Recent studies indicate the potential widespread occurrence of low-level concentrations of PPCPs and their metabolites in the aquatic environment, many of which are suspected endocrine disrupting chemicals (AWWA, 2001; Daughton and Ternes, 1999; Trussell, 2001). The persistence of EDCs and PPCPs in the aquatic environment is considered an emerging global environmental issue and potential concern for water quality and natural resources of southeastern Louisiana. International research efforts have been aimed at determining the sources and extent of EDC and PPCP contamination in surface water bodies. Currently, the U.S. Geological Survey is conducting a nationwide reconnaissance to determine the extent of PPCP contamination, which includes sampling the Mississippi River as far south as St. Francisville, Louisiana (http://toxics.usgs.gov). Other investigators are conducting research on the physiological and ecotoxicological effects of endocrine disrupting chemicals in water. However, very little research has been conducted regarding the occurrence of EDCs and PPCPs in waters of southeastern Louisiana and potential effects on the drinking water community, except for our preliminary research (Boyd et al., 2001; Grimm et al., 2001).

Drinking water treatment plants in the New Orleans area rely on the Mississippi River as their raw water source. As the conventional treatment methods are not specifically designed to remove possible contamination by EDC/PPCP compounds, the occurrence of these chemicals in the raw water source may pose a potential concern for the local drinking water community.

Progress Made to Achieve These Objectives

Dr. Siddhartha Mitra previously contributed to our research regarding EDCs/PPCPs in Lake Pontchartrain and the Mississippi River. He assisted our research by developing analytical methods for sample analysis using solid phase extraction (SPE). He selected compounds that represent a group of EDC/PPCP compounds that would be expected to persist in our water resources based on sales reported by pharmacists (see Table 1 for target compounds). Dr. Mitra developed draft "Standard Operation Procedures (SOP) for EDC/PPCP Isolation, Extraction and Analysis" to collect, extract, concentrate and derivatize samples and to quantify EDC/PPCP contaminants using GC/MS. Since Dr. Mitra completed his work in February, we have refined the SOP and are currently collecting samples from the Mississippi River, Lake Pontchartrain, the Jefferson Parish Water Treatment Plant and the Jefferson Parish Waste Water Treatment Plant for lab analysis.

From April to August 2001 Helge Reemtsma worked in the Civil and Environmental Engineering Department as a student intern. During this time we have conducted intense research to optimize the solid phase extraction procedure. We substituted our screening method with a quantitative method which provides a more efficient and reliable method for analyzing our target compounds. Our refinements included a few minor changes (flow rate, solvents volumes) as well as some important changes. We replaced the extraction disk from a less polar to a more polar SDB-XC empore disk. We also decided to bring every sample to dryness prior to derivatization in order to remove traces of water that occurred in the samples. In June we presented a poster at the AWWA conference in Washington, DC, summarizing the research conducted to date (Boyd et al., 2001)

In addition we have set up an experiment to determine the absorption capacity of activated carbon on organic compounds. To date we have determined the analytical method recovery rate for two of three compounds that we want to investigate. This method slightly varies from the method used for field. Bisphenol-A has a recovery rate of 80%. The recovery rate for naproxen is 32%. We expect to complete our experiments about adsorption capacity by the end of November.

From August 2001 to date, Mr. Reemtsma has been working in our lab as a Research Assistant for the CBR. He completed the development of our analytical procedure, which we are currently using to analyze raw and finished water samples. The method is reliable for all target compounds (except acetaminophen) and it shows trustworthy recovery rates of more than 50% for all compounds (except acetaminophen and caffeine). As the method shows slightly decreased recovery rates for the surrogate standards in these waters, compared to distilled water, we are currently investigating a way to increase recoveries by adding one more step to our procedure (remove large organic acids by passing the samples through a silica gel column).

In September we were able to use our method to analyze the first set of samples collected from the Mississippi River and Lake Pontchartrain. Two samples were collected from each surface water body and analyzed in our laboratory. We also analyzed water samples collected from the tap in our lab (see Table 2).

On October 10, 2001, we gave a talk on our research at the "2nd International Conference on Pharmaceuticals and Endocrine Disrupting Chemicals in Water" hosted by the NGWA in Minneapolis,

MN where we presented our analytical procedure and the first set of field sampling results (Grimm et al, 2001).

We are currently developing a draft paper regarding analytical methods, which we plan to submit to the journal *Environmental Science and Technology*. On November 12, 2001, Helge gave a talk about our research at seminar hosted by the "Institute for Earth and Ecosystem Science" (IEES) at Tulane University.

Other investigators who have contributed to this research include undergraduate students in the Department of Civil and Environmental Engineering plus participants in the LAMP (Louisiana Alliance for Minority Participation) program during summer 2001 as well as during fall semester 2001. From May to August 2001 Ms. Apryl Scott worked as an undergraduate student intern whose work was financed by the LAMP program. From August until now Ms. Scott works with us as a work-study student funded by the LAMP program. In August 2001 Apryl presented her work at the close of the summer program at Tulane. On November 16, 2001, Apryl will be presenting her recent research at Tuskegee University in Alabama.

The work Mr. Reemtsma has done from April to August contributes to his study in Germany (Environmental Engineering) which requires an internship in a study related subject. He is going to use the time he spends in our lab between August and December 2001 to finish his diploma (thesis) work.

Target Compound	Туре			
Clofibric Acid	Lipid regulator			
Estrone	Steroid			
17b- Estradiol	Steroid			
Ibuprofen	Analgesic			
Naproxen	Analgesic			
Acetaminophen	Analgesic			
Bisphenol- A	Fungicide and disinfectant			
Chlorophene	Fungicide and disinfectant			
Triclosan	Fungicide and disinfectant			
Fluoxetine	Antidepressant			
Caffeine	Human activity marker			

Table 1: target compounds

sampling site	Mississippi River		Lake Pontchartrain		Tulane tap water	
date	9/14/01	9/24/01	9/17/01	9/26/01	10/1/01	10/2/01
target compound						
Clofibric acid	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Ibuprofen	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Acetaminophen	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Acetaminophen-d4*	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Caffeine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Fluoxetine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Chlorophene	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Naproxen	37	39	107	22	N.D.	N.D.
Triclosan	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Bisphenol-A	5	N.Q.	N.Q.	N.Q.	N.D.	N.D.
Bisphenol-A-d14*	68.0%	75.0%	67.0%	67.0%	4.0%	14.0%
Estrone	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Estrone-d4*	103.4%	119.4%	88.3%	124.6%	2.3%	5.6%
17b-Estradiol	N.D.	N.D.	N.D.	N.Q.	N.D.	N.D.

^{*} SS std. Recovery, N.D. not detected, N.Q. not quantifiable

Table 2: field sampling results

Major Accomplishments

- Information about the occurrence and fate of EDCs/PPCPs in our local waters as well as in waste and drinking waters.
- Information about chlorinated byproducts in our local tap and wastewater and possible findings of EDC/PPCP contaminants as chlorinated byproducts.
- a publication about findings of our research on EDCs/PPCPs in southeastern Louisiana's surface water bodies, waste and drinking waters.
- Analytical methods for low-levels of contaminants for application to ONR/DOD biological agent monitoring

Publications, Manuscripts, Abstracts

Boyd, G.R., Grimm, D.A., 2001: "Occurrence of Pharmaceutical Contaminants and Screening of Treatment Alternatives for Southeastern Louisiana", *Annals of the New York Academy of Sciences*.

Presentations

Boyd, G.R. and Grimm, D.A., 2000: "Occurrence of Pharmaceutical Contaminants and Screening of Treatment Alternatives for the Lower Mississippi River", presented at the Emerging Issues Conference sponsored by the National Ground Water Association, Minneapolis, MN, June 7th 2000.

Boyd, G.R., Grimm, D.A. and H. Reemtsma, 2001: "Methods for Determining the Occurrence and Effectiveness of Treatment of PPCPs in Raw and Finished Water Supplies", presented at the American Water Work Association (AWWA) - Conference in Washington DC, June 2001

Grimm, D.A., Reemtsma, H. and Boyd, G.R., 2001: "Methods for Determining the Occurrence of PPCPs and EDCs in Waters of Southeastern Louisiana", presented at the National Ground Water Association (NGWA)- Conference in Minneapolis, MN, October 2001.

Meffert, D.J. A. Preimesber, J.A. McLachlan, and W. Toscano: Human and Ecological Species Integration for Functionally-based Assessments: A Bisphenol-A Case Study. Invited oral presentation at e.hormone 2001: the cutting edge of endocrine disruptor research symposium. Sponsored by the Center for Bioenvironmental Research, New Orleans, LA, October 18-20, 2001.

Intellectual Development

- 1. Student(s) Name: Helge Reemstma and Apryl Scott
- 2. Period of funding: April 2001-August 2001
- 3. Brief description of duties and responsibilities :see above

Useable Environmental Technologies

- 1. Title of technology product: N/A
- 2. **Description of technology product:** Analytical methods for detection of low-levels of pharmaceutically active compounds
- 3. **Utility/benefit/ROI/payoff of technology product:** Analytical methods for these emerging ubiquitous contaminants can be applied for detection of other biological agents of interest to the ONR and DOD.
- 4. **Timeline** (demonstration, validation, completion, etc.): Analytical methods will be refined and validated in the field by spring 2003
- 5. Partners (academia, industry, labs/centers, federal agency, etc.): ENWIN Utilities, Windsor, Ontario (a water utility utilizing methods developed in this project for water analysis)
- 6. Patents (applied for and issued)

Sedimentation and Resuspension Studies for the Mississippi River and Louisiana Coastal Environments

Principal Investigator:

Efstathios E. Michaelides, PhD.

Associate Dean, Engineering

Tulane University

Co-Investigator(s):

Laura J. Steinberg, PhD.

Assistant Professor

Civil and Environmental Engineering

Tulane University

Elia Eschenazi, PhD.

Associate Dean, Physics and Engineering

Xavier University of Louisiana

Reporting Period:

May 2001 - April 2002

Primary Objectives of Research Activities

To investigate the sedimentation and resuspension characteristics of single particles and flocs in aqueous solutions.

Progress Made to Achieve these Objectives

To investigate the sedimentation characteristics we have performed computational and experimental studies that yield information on the sedimentation behavior of single particles as well as clusters of particles. In the computational area we have developed a code, which utilizes the Lattice-Boltzman Method (LBM). This method is a recently developed technique in Computational Fluid Dynamics and enables us to consider the dynamics of single particles as well of large groups of particles. So far our code has been used to simulate the sedimentation process of up to 200 particles and we are extending its capabilities to 1000 particles. In the experimental area we have constructed a facility to measure the drag coefficients of spheres as well as irregular particles in viscous fluids. The range of the Reynolds numbers of the experiments is from 0.001 to 2000.

To investigate the resuspension characteristics we have performed computational studies that yield information on the sedimentation behavior of single particles as well as clusters of particles. In the computational area we have developed a code, which utilizes the Lattice-Boltzman Method (LBM). The method is the most recently developed technique in Computational Fluid Dynamics and enables us to consider the dynamics of single particles as well of groups of particles. Our code has been used to determine the lift force exerted by the flow on single particles present at the bottom of rivers and lakes as well as in determining the resuspension patterns and behavior of groups of particles.

Major Accomplishments

• We have completed a code based on the LBM to study the sedimentation and resuspension behavior of single particles and groups of particles.

N00014-99-1-0763

- We have determined the lift/resuspension force of a particle sitting in the bottom of a flow, which carries a suspension of similar particles.
- We have determined the sedimentation velocities and drag coefficients of particles settling in a chamber.
- We have determined the lift force on a moving particle that is close to a wall and, for light particles we have found out that they will not settle but will hover above the wall at an equilibrium position.
- We have determined the equilibrium position of light particles above a wall for spherical as well as prismatic particles.
- We have found out that the hydrodynamic lift forces exerted by a suspension of particles on a stationary particle are sufficient to cause the resuspension of the latter without any inter-particle collisions.
- We have found out that sedimentary particles strongly interact and form dynamic clusters. The characteristics of the sedimentation of the clusters are totally different than those of single particles.

Publications, Manuscripts, Abstracts

Feng, Z.-G. and Michaelides, E. E., "Drag coefficients of viscous spheres at intermediate and high Reynolds numbers," J. Fluids Eng., vol. 123, pp. 841-849, 2001.

Tsega, Y., Michaelides, E. E. and Eschenazi, E. V., "Particle dynamics and mixing in the frequency driven 'Kelvin cat eyes' flow," Chaos, vol. 11, pp. 351-358, 2001.

Feng, Z.-G. and Michaelides, E. E., "Inter-particle forces and lift on a particle attached to a solid boundary in suspension flow," <u>Physics of Fluids</u>, vol. 14, pp. 49-60, 2002.

Feng, Z.-G. and Michaelides, E. E., "Hydrodynamic Force on Spheres in Cylindrical and Prismatic Enclosures," <u>Int. J. Multiphase Flow</u>, vol. 28, pp. 479-496, 2002.

Michaelides, E. E., "Analytical Expressions," in <u>Transport Processes in Bubbles, Drops and Particles, eds.</u> DeKee and Chhabra, Taylor Francis, New York, 2002.

Michaelides, E. E., "Freeman Scholar Paper - Hydrodynamic force and heat/mass transfer from particles, bubbles and drops," <u>J. Fluids Eng.</u>, vol. 125, pp. 209-238, 2003.

Michaelides, E. E., "Models for multiphase flows," <u>von Karman Institute Lecture series</u>, editor J.-M. Buchlin, Brussels, Belgium, 2003.

Michaelides, E. E., "Introduction and basic equations for multiphase flow," <u>von Karman Institute Lecture series</u>, editor J.-M. Buchlin, Brussels, Belgium, 2003.

Michaelides, E. E., "Heat transfer and hydrodynamic force in dispersed multiphase flows," <u>von Karman Institute Lecture series</u>, editor J.-M. Buchlin, Brussels, Belgium, 2003.

Feng, Z.-G. and Michaelides, E. E., "Equilibrium position for a particle in a horizontal shear flow," <u>Int. J. Multiphase Flow</u>, vol. 29, pp. 943-957, 2003.

- Feng, Z.G. and Michaelides, E. E., "Fluid-particle interactions and resuspension in simple shear flow," ASCE J. Hydraulic Eng., in print, 2003.
- Xu, Z-J., and Michaelides, E. E., "A numerical simulation of the Boycott effect," <u>Chem. Eng. Communications</u>, accepted for publication, 2003.
- Tran-Cong, S., Feng, Z.-G. and Michaelides, E. E., "A new model for the transport of suspended sediment," <u>ASCE J. Hydraulic Eng.</u>, accepted for publication, 2003.
- Xu, Z-J., and Michaelides, E. E., "The effect of particle interactions on the sedimentation process of non-cohesive particles," <u>Int. J. Multiphase Flow</u>, vol. 29, pp. 959-982, 2003.

Presentations

- Feng, Z.-G. and Michaelides, E. E. "Fundamental studies on the resuspension of particles," APS-DFD annual meeting, San Diego CA November 2001.
- Kartushinski, A. and Michaelides, E. E. "Inter-particle collisions-Two-fluid model for closure," 10-th Workshop on Two-Phase Flow Predictions, Erlangen, Germany, April 2002.
- Michaelides E. E., Zhi-Gang Feng and Tran-Cong, S., "Sediment transport models with coupled momentum and solids mass transport, 5th Int. Conf. On Hydro-science and Engineering, Warsaw, Poland, July 2002.
- Michaelides E. E. and Feng Z.-G., "History terms in the heat and mass transfer equations of particles," Proc. of the 12th Intern. Heat Transfer Conf., Grenoble, France, August 2002.
- Xu Z.-J. and Michaelides E. E., "Lattice Boltzmann simulation of the sedimentation process with non-cohesive particles," Particle Fluids Interactions-VI, Barga, Italy, August 2002. (Keynote Lecture)
- Feng Z.-G. and Michaelides E. E., "Fluidization and resuspension of particles in simple shear flow," Particle Fluids Interactions-VI, Barga, Italy, August 2002.
- Michaelides, E. E., "Hydrodynamic force and heat/mass transfer from particles, bubbles and drops," ASME, Int. Mechanical Engineering Conference and Exhibition, New Orleans, LA, November, 2002. (Plenary Lecture-The Freeman Scholar Lecture)
- Michaelides, E. E. and Xu, Z.-J. "Particle interactions during sedimentation," APS-DFD Annual meeting, Dallas TX, November 2002.
- Tsega, Y., Michaelides, E. E. and Eschenazi, E., "Particle dynamics and mixing in the frequency driven 'Kelvin cat eyes' flow," 4th International Conference on Multiphase Flow, New Orleans, LA, May 2001.

Intellectual Development

1. Student(s) Name: Zhi-Gang Feng, post-doctoral student

Sabine Tran-Cong, post-doctoral student Alexander Kartushinski, research associate

Xujia Xu, Ph.D. Student, Mechanical Engineering

Jim Martin, Ph. D. Student, Civil and Environmental Engineering

Swirvine Niyendra, Ph. D. Student, Civil and Environmental Engineering

Lorenzo Craig, BS student, Physics-Xavier Michelle Wyche, BS student, Physics-Xavier

2. Funding Period: April 2001 to April 2002

3. **Duties and Responsibilities** Dr. Michaelides was in charge of the direction of the research team and the advisement of the graduate and undergraduate students. Dr. Feng assisted with the computational aspects and helped in the advisement of Messers Xu, Craig and Wyche. Dr. Tran-Cong assisted with the experimental part of the research and helped with the advisement of Messers Martin and Nyiyendra.

Useable Technologies: The LBM computer code can be used for the simulation of particle flow.

COAMPS for Coastal Urban Transport Studies - Developing Infrastructure and Testing Models

Principal Investigator:

David J. Sailor, Ph.D. Associate Professor

Dept. of Mechanical Engineering

Tulane University

Reporting Period:

May 2001 – April 2002

Summary

In discussions with representatives of the Office of Naval Research (Simon Chang and Ron Ferek) the PI identified a unique opportunity for future research and development efforts related to coastal urban applications of the COAMPS mesoscale atmospheric modeling system currently in use by the U.S. Navy. Specifically, the PI has been developing unique modeling improvements for another leading mesoscale model (MM5 from the National Center for Atmospheric Research) that will allow enhanced predictive capability for atmospheric flow and heat transfer in and around metropolitan regions. As a follow-up to his July 2000 meeting at ONR the PI put in place the necessary Memorandum of Agreement (MoA) with the Naval Research Laboratory to obtain full access to the source code and related data and documentation for COAMPS. A full copy of the COAMPS code has been downloaded to the PI's Linux workstations, has been compiled, installed, and tested.

Primary Objectives of Research Activities

The goals of this one-year project were relatively straight-forward, and will all lead to an enhanced capability for the PI to participate in ONR research in the area of mesoscale meteorological modeling.

These goals are:

- Successfully install and compile the COAMPS code on the PI's computers
- Develop a baseline simulation using COAMPS and validate its performance

After having completed these goals the PI is in a position to propose cutting-edge research projects involving COAMPS. The first such project will involve enhancing the ability of COAMPS to represent surface characteristics, anthropogenic heat flux, and radiative transport in the complex urban environment.

Progress Made to Achieve these Objectives

The initial goal of this project was to install COAMPS on the existing IBM RS6000 machine (oahu.me.Tulane.edu) that is part of Dr. Sailor's computational infrastructure. The goal of installing COAMPS on Linux machines was a secondary goal that was initially deemed to be higher risk. However, in consultation with the Naval Research Laboratory (NRL) and their COAMPS documentation it was decided that a Linux installation would, in fact, be more straightforward. Hence, the order of priorities shifted and we have successfully installed all COAMPS components on the recently purchased Linux system.

Two baseline simulations have been conducted. These are September 1-3 and January 26-28, 1999. The domain for these simulation covers the western continental United States (conus). All necessary input files

for the corresponding MM5 simulation have been obtained and now reside in a Data partition on our group's main MM5 platform (oahu.me.Tulane.edu).

Major Accomplishments

- Signed Memorandum of Agreement (MoA) with Naval Research Laboratory allowing access to COAMPS model and data
- Downloaded COAMPS Utility, Analysis, and Forecast code modules
- Obtained Linux machine for simulations and installed necessary Fortran compilers (PGF)
- Installed all COAMPS components on Linux machine (lanai.me.Tulane.edu)
- Successfully installed and debugged several of the utility modules including the domain definition tool and the model input analysis program.
- Submitted a letter of intent to write a competitive proposal for funding no word received to date regarding ONR's interest.

Publications, Manuscripts and Abstracts

This is an infrastructure-building project that is not anticipated to yield peer-reviewed publications in the first year. However, we have the intention, once the entire COAMPS model is up and running of simultaneously developing our model urbanization tools for both COAMPS and MM5 and presenting comparison results in an archival journal or at a major conference (e.g. AMS annual meeting).

Presentations

None (see above)

Intellectual Development

- 1. Student Name: Lu Lu
- 2. **Period of funding:** October 2001 April 2002
- 3. **Responsibilities:** Assisting in gathering relevant data for model initialization and validation. Also gathering necessary data for development of anthropogenic heat profiles.

Useable Environmental Technologies

- 1. **Technology Product:** An urbanized mesoscale model (anticipated, with additional support)
- 2. **Description:** This product will involve several model enhancements to the existing version of COAMPS. Specifically, these enhancements will improve the way in which the model represents radiative exchange in the urban canopy, will add the capability to model effects of anthropogenic heat generation in cities (waste heat and respiration heat), and will investigate other model enhancements.
- 3. **Utility of Product:** The value of this product is that it will allow for significant improvements in all aspects of short-term weather forecasts in and near urban domains. As a result, the urbanized model will be better able to capture the meteorological aspects of the urban heat island effect, and fate and transport of pollutants in urban domains.

- 4. **Timeline:** This product is envisioned as the follow-on to the present project and would involve an additional investment of ONR resources for approximately 3 years.
- 5. **Partners:** The EPA has an interest in improving the capabilities of models over urban areas as a means of improving the capabilities of photochemical air quality models.
- 6. Patents: N/A

APPENDIX B.

PUBLICATIONS, MANUSCRIPTS, ABSTRACTS, PRESENTATIONS

Publications, Manuscripts, Abstracts

Publications

- **Allison, M.A.** and Neill, C.F., 2002. Accumulation rates and stratigraphic character of the modern Atchafalaya River prodelta, Louisiana. *Transactions of the Gulf Coast Association of Geological Societies*, 52:1031-1040.
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Satula, M., Bianchi, T. and **McKee**, **B**. Effect of Seasonal Sediment Storage and Diagenesis in the Lower Mississippi River bed on Bioavailability of Particulate Phosphorus Flux to the Gulf of Mexico. 2001 Aquatic Sciences Meeting sponsored by the American Society of Limnology and Oceanography; Albuquerque NM.

Tsega, Y., **Michaelides, E. E.** and Eschenazi, E., "Particle dynamics and mixing in the frequency driven 'Kelvin cat eyes' flow," 4th International Conference on Multiphase Flow, New Orleans, LA, May 2001.

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Xu, Y. and Marcantonio, F. (2001) Sr and trace metals in the Mississippi River mixing zone, *Geophys. Union Trans.*, 82, F648.

Xu Z.-J. and **Michaelides E. E.**, "Lattice Boltzmann simulation of the sedimentation process with non-cohesive particles," Particle Fluids Interactions-VI, Barga, Italy, August 2002. (Keynote Lecture)

APPENDIX C.

USEABLE TECHNOLOGIES

Summary of Useable Technologies

Environmental Signals and Sensors

BLAKE, D.

- 1. **Title of technology product**: Immunosensor for deployment in AUV; Recombinant antibodies for environmental analysis
- 2. **Description of technology product**: This antibody-based biosensor will be able to automatically collect and analyze 5 separate samples after installation in an autonomous underwater vehicle or immobilized buoy (EARS);
- 3. **Utility/benefit/ROI/payoff of technology product**: A self-contained, automated immunosensor will have the capability to detect very low concentrations of environmental contaminants and/or chemical and biological weapons in surface waters.
- 4. **Timeline**: An assay that detects nanomolar levels of EDTA, the first analyte to be developed for this instrument, has been established. Transfer of the assay to the immunosensor will begin when Sapidyne has corrected the defects in the optical components of the instrument.
- 5. **Partners**: Sapidyne Instruments (Boise, ID) is constructing the immunosensor and our laboratory is working closely with them to coordinate the development of biological reagents with the development of the instrument. The Blake laboratories also have strong ties with Dr. Fran Ligler's laboratory at the Naval Research Laboratory in Washington D.C. James Delehanty, who recently received his Ph.D. in Diane Blake's laboratory, is now an NRC fellow in Dr. Ligler's laboratory.

Ecosystem Monitoring and Assessment

MEFFERT, D.J.

- 1. **Title of technology product:** Integrated autonomous immunosensor and autonomous underwater vehicle system.
- 2. **Description of technology product:** This system will enhance real-time biosensor deployment for environmental compliance and ultimately biologic warfare detection.
- 3. **Utility/benefit/ROI/payoff of technology product:** Enhanced real-time biosensor deployment for environmental compliance and, ultimately biologic warfare detection.
- 4. **Timeline (demonstration, validation, completion, etc.):** AUV/biosensor integration to be completed in August 2004. Biosensor to be deployed on AUV or stationary buoys and AUV's subsequent to August 2004, pending acquisition of REMUS AUV.
- 5. Partners (academia, industry, labs/centers, federal agency, etc.): Naval Oceanographic Office, Tulane and Xavier Universities, Sapidyne Instruments, Boise, Idaho, COTS Technology, LLC, New Orleans, LA, Woods Hole Oceanographic Institute, Woods Hole, Massachusetts
- 6. Patents (applied for and issued): Patents will be applied for by partners

C2

SAILOR, D.

- 1. Technology Product: An urbanized mesoscale model (anticipated, with additional support)
- 2. Description: This product will involve several model enhancements to the existing version of COAMPS. Specifically, these enhancements will improve the way in which the model represents radiative exchange in the urban canopy, will add the capability to model effects of anthropogenic heat generation in cities (waste heat and respiration heat), and will investigate other model enhancements.
- 3. Utility of Product: The value of this product is that it will allow for significant improvements in all aspects of short-term weather forecasts in and near urban domains. As a result, the urbanized model will be better able to capture the meteorological aspects of the urban heat island effect, and fate and transport of pollutants in urban domains.
- **4. Timeline:** This product is envisioned as the follow-on to the present project and would involve an additional investment of ONR resources for approximately 3 years.
- **5. Partners:** The EPA has an interest in improving the capabilities of models over urban areas as a means of improving the capabilities of photochemical air quality models.

APPENDIX D.

INTELLECTUAL DEVELOPMENT

Intellectual Development

		Intellectual Development	
Student Name	Level	<u>Institution</u>	<u>Mentor</u>
Carlson, John	Graduate	Tulane University	Scott Michael Ph.D.
Craig, Lorenzo	Graduate	Tulane University	Elia Eschenazi Ph.D.*
Delahanty, James	Graduate	Tulane University	Diane Blake Ph.D.
Feng, Zhi-Gang	Graduate	Tulane University	Efstathios Michaelides Ph.D.
Frigo, Dan	Graduate	Tulane University	John McLachlan Ph.D.
Galler, John	Graduate	Tulane University	Mead Allison Ph.D.
Gibbs, Monique	Graduate	Tulane University	Elia Eschenazi Ph.D.*
Gonzalez, Jo	Graduate	Universidad Metropolitana, San Juan, PR	Scott Michael Ph.D.
Kartushinski, Alexander	Graduate	Tulane University	Efstathios Michaelides Ph.D.
Kriegel, Alison	Graduate	Tulane University	Diane Blake Ph.D.
Lu, Lu	Graduate	Tulane University	David Sailor Ph.D.
Martin, Jim	Graduate	Tulane University	Efstathios Michaelides Ph.D.
Neill, Ciara	Graduate	Tulane University	Mead Allison Ph.D.
Niyendra, Swirvine	Graduate	Tulane University	Efstathios Michaelides Ph.D.
Reemstma, Helge	Graduate	Tulane University	Doug Meffert Ph.D.
Stewart, Mike	Graduate	Tulane University	Brent McKee Ph.D.
Toro, Esteban	Graduate	Universidad de los Andes, Bogota, Colombia	Scott Michael Ph.D.
Tran-Cong, Sabine	Graduate	Tulane University	Efstathios Michaelides Ph.D.
Vincent, Shawn	Graduate	Tulane University	Scott Michael Ph.D.
Wyche, Melodie	Graduate	Xavier University	Elia Eschenazi Ph.D.*
Wysocki, Laura	Graduate	Tulane University	Brent McKee Ph.D.
Xu, Xujia	Graduate	Tulane University	Efstathios Michaelides Ph.D.
Xu, Yingfeng	Graduate	Tulane University	Franco Marcantonio Ph.D.
Bogin, Gregory	Undergraduate	Xavier University	Kathleen McLoud Ph.D.
Brown, Herbert	Undergraduate	Xavier University	Kathleen McLoud Ph.D.
Lourido, Sebastian	Undergraduate	Tulane University	Scott Michael Ph.D.
Myers, Paula	Undergraduate	Xavier University	Kathleen McLoud Ph.D.
Philip, Cornel	Undergraduate	University of the Virgin Islands	Diane Blake Ph.D.
Robinson, Merideth	Undergraduate	Xavier University	Kathleen McLoud Ph.D.
Scott, Apryl	Undergraduate	Tulane University	Doug Meffert Ph.D.
Vigh, Katinka	Undergraduate	Tulane University	Matthew Burrow Ph.D.
Aregbesola, Juliet	SPRITE	Tulane University	William Halford Ph.D.
Cody, William	SPRITE	Tulane University	Michael Schurr Ph.D.
Collins, Ashte'	SPRITE	Tulane University	Sanda Clejan Ph.D.
Davis-Moliere, Shakia	SPRITE	Tulane University	Karen Friday Ph.D.
Gamble, Lena	SPRITE	Tulane University	Sanda Clejan Ph.D.
Stepter, Alayna	SPRITE	Tulane University	Scott Michael Ph.D.
		•	

 $[\]star$ Tulane/Xavier collaborative mentoring program 3+2+2, leading to a master's degree in Engineering.

APPENDIX E.

HISTORICAL DOCUMENTS

- **❖** BAA
- ❖ Award/Modification Letter(s)
- Timeline
- SF 298 Cover Sheet



Solicitation Number: BAA 00-018

Due Date: 8/31/2001 **Classification:** A **Type:** Procurement

Agency:

Office of Naval Research 800 North Quincy Street Arlington, VA 22217-5660

TITIE: LONG RANGE SCIENTIFIC AND TECHNOLOGY PROGRAM

Point of Contact:

Beverly S. Harris, ONR OOST (703) 696-5419

SYNOPSIS:

The Office of Naval Research (ONR) is interested & shy; in receiving proposals for Long-Range Science and Technology Projects which offer potential for advancement and improvement of Navy and Marine Corps operations. Readers should note that this is an announcement to declare ONR's broad role in competitive funding of meritorious research across a spectrum of science and engineering disciplines. No request for proposal (RFP), solicitation or other announcement of this opportunity will be made. This announcement will be open for approximately one year from date of publication until replaced by a successor Broad Agency Announcement (BAA). Proposals may be submitted any time during this period. Awards may take the form of contracts, grants, cooperative agreements, or other transactions.

Proposal submission is not restricted in any way to any particular entity. Historically Black Colleges and Universities, Minority Institutions, Tribal Colleges and Universities, and small, HUBZone small, small disadvantaged, and women owned small businesses are encouraged to participate.

PROPOSAL PREPARATION

Before preparing proposals, potential offerors are strongly encouraged to contact the ONR Program Officer whose program best matches the offeror's field of interest as listed in the Science and Technology section of the ONR Home Page accessible through World Wide Web at http://www.onr.navv.mil. THE ONR LONG RANGE BAA 00-018 IS A COMPETITIVE SOLICITATION. PROPOSALS SUBMITTED IN RESPONSE TO THIS SOLICITATION MUST CITE BAA 00-018 (PREFERABLY ON THE COVER PAGE). Proposals should also be accompanied by a completed certification package which can be accessed on the ONR Home Page at Contract & Grants How to Submit a Proposal. For grant proposals (normally submitted by universities) the certification package is entitled Certifications for Grants and Agreements. For contract proposals (normally submitted by commercial contractors) the certification package is entitled Representations and Certifications for Contracts.

(Documents with .doc extension may require Microsoft Word, or the Word viewer)

WHERE TO SUBMIT PROPOSALS

Proposals should be sent to the attention of the ONR Program Officer whose program best matches the offeror's field of interest at the Office of Naval Research, 800 North Quincy Street, Arlington, VA 22201.

POINTS OF CONTACT

General questions can be submitted to Beverly S. Harris ONR OOST (703) 696-5419, email harrisb@onr.navv.mil

Questions of a technical nature should be submitted to the ONR Program Officer whose program best matches the offeror's field of interest as listed in the Science and Technology section of the ONR Home Page accessible through World Wide Web at http://www.onr.navy.mil.

DEPARTMENT OF DEFENSE HIGH PERFORMANCE COMPUTING PROGRAM

The DoD High Performance Computing Program (HPCMP) furnishes the DoD S & T and DT & E communities with use-access to very powerful high performance computing systems. Awardees of ONR contracts/grants may be eligible to use HPCMP assets in support of their funded activities if the ONR Program Officer approval is obtained and if the security/screening requirements are favorably completed. Award decisions will be based on a competitive selection of proposals resulting from a scientific/technical review. Additional information and an application may be found at http://www.hpcmo.hpc.mil/.

EVALUATION OF PROPOSALS

Evaluations will be conducted using the following evaluation criteria: (1) overall scientific, technical and/or socio-economic merits of the proposal; (2) potential naval relevance and contributions of the effort to the agency's specific mission; (3) the offeror's capabilities, related experience, facilities, techniques or unique combinations of these which are integral factors for achieving the proposal objectives; (4) the qualifications, capabilities and experience of the proposed Principal Investigator, team leader and key personnel who are critical in achieving the proposal objectives; (5) realism of the proposed cost and availability of funds.

INDUSTRY-ACADEMIA PARTNERING

ONR highly encourages partnering among industry and academia with a view to speeding the incorporation of new science and technology into fielded systems. Proposals that utilize industry-academic partnering which enhances the development of novel S & T advances will be given favorable consideration.

SOCIO-ECONOMIC MERIT EVALUATION

For awards made as contracts, the socio-economic merits of each proposal will be evaluated based on the commitment to provide meaningful subcontracting opportunities for Small Businesses, HUBZone small businesses, Small Disadvantaged Business, Woman-Owned Small Business Concerns, Historically Black Colleges and Universities, Minority Institutions, and Tribal Colleges and Universities. In addition, contract proposals that exceed \$500,000, submitted by all but small business concerns, must be accompanied by a Small Business Subcontracting Plan in accordance with FAR 52.219-9. The North American Industry Classification System (NAICS) code for this solicitation is 541710 (which corresponds with the standard industrial classification code of 8731) with the small business size standard of 500 employees.

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Author: 02 Web Author

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Modified: 3 November 2001



AWARD/ MODIFICATION

3a. ISSUED BY: OFFICE OF NAVAL RESEARCH BALLSTON CENTRE TOWER ONE 800 NORTH QUINCY STREET ARLINGTON VA 22217-5660

1. INSTRUMENT TYPE 3h CEDA: Grant 12.300 2. AUTHORITY: 3c. DUNS NUMBER: 10 USC 2358, 31 USC 6304 4. AWARD NO.; 5. MODIFICATION NO.: 6. MODIFICATION TYPE: 7. PR NO PAGE N00014-99-1-0763 P00003 01PR08429-00 Renewal 1 of 5 8. ACTIVITY/AGENCY PROPOSAL NO .: 9. RECIPIENT PROPOSAL NO.: 10. PROPOSAL DATE: 11. ACTIVITY TYPE 12. PROGRAM TYPE: 01342 -- 0208 N/A 19-JAN-2001 Research N/A 13. ISSUED TO 13b CAGE 13c. EDI/EFT NUMBER: 14. REMITTANCE ADDRESS (IF DIFFERENT FROM BLOCK 13): 13a, ADDRESS: 4B966 Same as block #13 TULANE UNIVERSITY OFFICE OF RESEARCH AND PROJECT ADMINISTRATION 327 GIBSON HALL NEW ORLEANS, LA 70118-5698 13d. BUSINESS OFFICE CONTACT: Kozar, Kathleen 13e. TELEPHONE NUMBER: 13f. EMAIL ADDRESS: (504) 5885207 15. RESEARCH TITLE AND/OR DESCRIPTION OF PROJECT AND/OR PROPOSAL TITLE: Integrated Bioenvironmental Hazards Research Program 16. FL NDING ACTIVITY/AGENCY SHARE RECIPIENT SHARE TOTAL 17. CURRENT FUNDING PERIOD N/A THROUGH \$5,591,000.00 PREVIOUSLY OBLIGATED: \$.00 \$5,591,000.00 N/A OBLIGATED BY THIS ACTION: \$1,900,000.00 \$.00 \$1,900,000.00 TOTAL OBLIGATED ON AWARD: \$7,491,000.00 \$7,491,000.00 18. PERIOD OF PERFORMANCE \$.00 01-MAY-1999 FUTURE FUNDING: \$.00 \$.00 \$.00 THROUGH 30-APR-2002 GRANT TOTAL: \$7,491,000.00 \$.00 \$7,491,000.00 19. ACCOUNTING AND APPROPRIATION DATA: See attached Financial Accounting Data Sheet(s) 20a. PRINCIPAL INVESTIGATOR/RECIPIENT 21. TECHNICAL REPRESENTATIVE 21b. CODE: TECHNICAL REPRESENTATIVE: 21a NAME: Joe L. Brumfield ONR 342 (PI) John A. McLachlan 21c. ADDRESS OFFICE OF NAVAL RESEARCH BALLSTON CENTRE TOWER ONE 800 NORTH QUINCY STREET ARLINGTON VA 22217-5660 20t "ELEPHONE NUMBER: 120c. EMAIL ADDRESS: 21d. TELEPHONE NUMBER: 21e. EMAIL ADDRESS: (504) 5856910 (703) 6964057 brumfijeonr.nevy.mil 22. AWARDING OFFICE CONTACT 22b. CODE: 23a. ADMINISTRATIVE OFFICE: 23b. CODE: 22a : AME: Julia M. Gallmon ONR 252 N66020 22c. ADDRESS: OFFICE OF NAVAL RESEARCH REGIONAL OFFICE ATLANTA OFFICE OF NAVAL RESEARCH 100 ALABAMA STREET NW SUITE 4R15 BAILSTON CENTRE TOWER ONE ATLANTA GA 30303-3104 860 NORTH QUINCY STREET Fax: (404) 5621610 ARLINGTON VA 22217-5660 22d. TELEPHONE NUMBER: | 22e. EMAIL ADDRESS: (703) 6962609 gallmo;@onr.navv.mil 24. SUBMIT PAYMENT REQUEST TO: 25a. PAYING OFFICE: 25b. CODE: 26a. PATENT OFFICE: 26b. CODE: N68892 N00014 Same as block #23a DFAS CHARLESTON, SC OFFICE OF NAVAL RESEARCH ATTN: ONR 00CC BALLSTON CENTRE TOWER ONE 800 NORTH QUINCY STREET ARLINGTON VA 22217-5660



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REPORT DOCUMENTATION PAGE

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